The present study was conducted to evaluate the individual and combined toxicopathology of chlorpyrifos and cypermethrin in broiler chickens. Broiler chicks (80), day-old, were divided into four groups. Group T1 served as control, whereas Group T2 was treated with chlorpyrifos @ 50 mg/kg feed, T3 with cypermethrin @ 200 mg/kg feed and T4 with chlorpyrifos @ 50 mg/kg + cypermethrin @ 200 mg/kg feed for 4 weeks. During 5th week, the dietary treatment was withdrawn and observed for withdrawal effect. At the end of 4th and 5th week, six birds from each group were sacrificed and examined for gross and histopathological changes. Microscopically, liver showed mild to extensive granular and vacuolar changes, necrosis and lymphoid aggregation. Granular and vacuolar changes in tubular epithelium along with necrosis of tubules and detachment of tubule from the basement membrane were observed in kidneys. Heart showed varying degrees of degenerative changes in cardiac muscle fibres. Intestine showed diffuse degenerative changes along with necrosis and fusion of villi. Brain showed mild to moderate degenerative changes and neuronophagia. Sciatic nerve showed degenerative changes along with infiltration of mononuclear cell and swelling of axons. The lesions were more prominent in combined toxicity group. However, at 7th day post withdrawal, restoration towards normal parenchyma was observed in visceral organs. Ultra structure of liver at 4th week revealed pronounced pathological lesions in cell organelles. Thus it can be concluded that chlorpyrifos and cypermethrin induced adverse toxic effect individually as well as in combination.

Keywords: Broiler, Chlorpyrifos, Cypermethrin, Toxicopathology, Ultra structure
Clinical observations: Throughout the 4 weeks of experimentation, each group was maintained with identical management and hygienic conditions. From day 0, a specific dietary treatment was given to each group. Group T1 served as control, whereas Group T2 was fed with chlorpyrifos @ 50 mg/kg feed, Group T3 with cypermethrin @ 200 mg/kg feed and Group T4 with combination of chlorpyrifos @ 50 mg/kg and cypermethrin @ 200 mg/kg feed for 4 weeks. Broiler chicks were fed with ad lib. dietary treatment and water was given ad lib. throughout the experimental period of 4 weeks. During 5th week, the respective dietary treatment was withdrawn and all the groups were fed with normal control diet for one week. All the birds were maintained with identical management and hygienic conditions.

Histopathological preparation: At the end of 4th week and 5th week (at 7th day post withdrawal) of experiment, six birds from each group were selected randomly and were sacrificed by cervical dislocation. Detailed postmortem examination was conducted and gross lesions were recorded. Tissues of liver, heart, kidney, brain, intestine and sciatic nerve were fixed in 10% buffered formal saline solution and after proper fixation, tissues were dehydrated by graded doses of alcohol, cleared in xylene and embedded in paraffin (58–60°C). Sections of 4–6 micron thickness were then cut and stained by routine Hematoxylen and Eosin stain (Luna 1968).

Ultra structural preparation: At the end of 4th week, tissues of liver from all groups were collected and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde at 4°C for 6–8 h which was then followed by post-fixation in 1% Osmium tetroxide. After that tissues were dehydrated in acetone and embedded in araldite. Ultrathin sections (70–90 nm) were cut on the ultramicrotome and stained with alcoholic uranyl acetate and alkaline lead citrate, washed gently with distilled water and observed under a transmission electron microscope at an operating voltage of 200 kV (Spurr 1969).

RESULTS AND DISCUSSION

Gross and histopathological observations: Grossly, T2 group exhibited lesions of swollen pale coloured liver with pin point hemorrhages and congested and edematous lungs. Intestine showed haemorrhages along the length of duodenum with thickened duodenal mucosa. Group T3 showed similar lesions of mild intensity. Group T4 showed similar lesion of moderate intensity with pale swollen kidneys, swollen spleen with congestion and yellowish colour swollen liver. Comparatively more toxic lesions in T4 group indicate additive effect of chlorpyrifos and cypermethrin when fed in combination. These degenerative changes in the vital organs due to the toxic influences of these compounds might be the reason for gross pathological observations recorded during present investigation. Present findings are in agreement with Kammon et al. (2010) and Kumar et al. (2012) for chlorpyrifos toxicity and Baba et al. (2015) for cypermethrin toxicity.

Microscopically, sections of liver, heart, kidney, intestine, brain and sciatic nerve from control group birds showed normal histoarchitecture with mild blood vessel congestion at the end of 4th week as well as at 7th day post withdrawal.

The light microscopic examination of liver from Group T2 showed moderate to extensive granular and vacuolar changes in hepatocytes, increased sinusoids, focal areas of lymphoid aggregation, mononuclear cell infiltration and necrosis suggested toxicity lesions and the onset of immunological response by the host. Group T3 also showed similar type of mild to moderate degenerative changes. Group T4 showed similar degenerative changes with that of Group T2 along with swelling of hepatocytes with nucleus pushed towards periphery, proliferation of Kupffer cells and mild to moderate fatty changes. These findings corroborates with Kumar et al. (2012) regarding chlorpyrifos toxicity in broilers. Liver is site of metabolism and bioactivation for toxicants, where toxicants are rapidly bioactivated. The inhibitory effect of chlorpyrifos and cypermethrin on total adenine triphosphate activity may disturb active transport of Na+, K+ and Ca++ ions thus injured hepatotyes causing various degenerative changes (Khan et al. 2009). Increased focal areas of lymphoid aggregation and necrosis might be due to damage and fragmentation of genomic DNA, leading to liver injuries and necrosis (Abdou et al. 2012). The molecular mechanism of tissue toxicity by chlorpyrifos is mainly due to the generation of reactive oxygen species which causes membrane damage of cell resulting into degeneration to necrotic changes in different visceral organs (Kumar et al. 2012). Kulthe et al. (2018) observed similar lesion in subacute chlorpyrifos toxicated quails and suggested hepatic and renal injury either by generating free radicals or by altering enzymatic or non enzymatic antioxidant defense system of the body.

Heart of Groups T2 and T3 birds showed extravasation of erythrocytes in between cardiac muscle fibres with polymorphonuclear cells infiltration suggesting inflammatory changes which might be due systemic toxicity of toxins or due to the effect of its metabolites (Baba et al. 2015). Group T4 showed prominent infiltration and aggregation of mononuclear and polymorphonuclear cells along with myofibril necrosis leading to widening of muscle fibres indicating more adverse effect in combination. The multiple effects of chlorpyrifos had been reported on the target cells which include generation of reactive oxygen species and induction of intracellular oxidative stress which ultimately causes disruption of the normal cellular development and differentiation resulting in significant histopathological alterations in different organs (Dettbarn 2006).
Kidney of Groups T2 and T3 birds exhibited mild to moderate granular and vacuolar changes in the tubular epithelium, glomerular degeneration and tubular necrosis along with detachment of tubular epithelium from the basement membrane. However, Group T4 showed moderate to extensive granular and vacuolar changes in the tubular epithelium with foci of tubular necrosis and detachment of tubular epithelium from the basement membrane. Tubular lesion observed suggested direct toxic effect of chlorpyrifos and cypermethrin given either individually or in combination. The presence of necrosis may be related to the depletion of ATP, leading to the death of cell (Shimizu et al. 1996). The degenerative changes observed are in accordance with previous findings of chlorpyrifos and cypermethrin toxicity in broilers (Kammon et al. 2011, Mamun et al. 2014, Begum et al. 2015, Chandana et al. 2015, Islam and Hoque 2015). The lesions thus suggested nephrotoxic effect of chlorpyrifos and cypermethrin which might be due to leakage of proteins that causes tubular necrosis. Kidney plays an important role in the detoxification for many xenobiotics and is frequently susceptible to the nephrotoxic effects (Kammon et al. 2010) which might be the reason for present observations.

Intestine from Groups T2 and T4 birds showed loss of branching pattern, diffuse degeneration, necrosis at the tip of villi and desquamation and sloughing of epithelial cells in the lumen. Most of the section showed fusion of villi with increase in lymphoid populations and inflammatory cells at lamina propria and submucosa. The present findings thus demonstrated direct toxic injury to intestinal mucosa leading to inflammatory response (Baba et al. 2015). The observed lesions might have some irritant effect of chlorpyrifos on the epithelial surface of the intestine. The lesions observed in intestine had resemblance with earlier finding (Krishnamoorthy et al. 2007). Sections from Group T3 birds showed long slender villi, necrosis at tip of villi and loss of branching pattern with mild to moderate diffuse degenerative changes. Hedau et al. (2018) also recorded toxic pathological effect of chlorpyrifos on digestive tract including proventriculus, gizzard and intestine given either individually or in combination with acetamiprid in broilers. However the lesions were more intense in combination group suggesting additive effect of pesticides and corroborating the present findings.

Brain from Group T2 birds showed vacuolar degeneration in the brain cytoplasm, moderate degenerative changes in neurons and increase in Virchow-Robin spaces. Liquefactive necrosis was observed in two sections. The present observation corroborates with previous findings of chlorpyrifos toxicity in broilers (Kammon et al. 2010). Group T3 also showed somewhat similar changes with mild intensity and increase in number of glial cells. Group T4 showed prominent satellitosis and neuronal degeneration. The present findings of neurotoxicity of chlorpyrifos are in agreement with earlier finding in broilers (Kumar et al. 2012) and it might be due to the ability of chlorpyrifos and cypermethrin to cross the blood-brain barrier and high lipid solubility due to presence of choline group (Ahmad et al. 2015). Many widely used agricultural chemicals induce oxidative damage in various systems of the body such as in dopaminergic cells of the brain by modulating the antioxidant defense system. The low SOD activity in brain and heart tissues as compared to its activity in the liver tissue, favours accumulation of oxygen free radicals which may lead to tissue damage as a result of oxidative binding of key intracellular molecules containing thiol groups and lipid peroxidation of biological membranes, which might be of greatest importance in the cytotoxicity of pesticides (Alhifi 2010).

Sections of sciatic nerve from Group T2 showed focal areas of mononuclear cell infiltration in epineurium, moderate separation of nerve fibres, swelling of axons and mild degeneration of Schwann cells. Group T3 birds also exhibited mild degenerative changes in Schwann cells and mononuclear cell infiltration. Similar types of lesion with edema and moderate to high intensity were observed in Group T4. Similar degenerative changes in sciatic nerve were also reported by earlier researchers about chlorpyrifos and cypermethrin toxicity (Kammon et al. 2010, Suzan 2012). Microscopic lesions in different organs are thus suggestive of possibly severe toxic injury to capillary endothelium which may cause development of widespread vascular lesions in various organs (Yadav et al. 2018).

At 7 th day post withdrawal, grossly, birds from all treatment group exhibited pale colour liver with moderate swelling, mild congested lungs and pale colour kidneys indicated mild restoration of lesions after withdrawal of toxicants. Microscopically, liver from Groups T2 and T4 birds revealed mild to moderate granular and vacuolar changes in the hepatocytes along with necrobiosis and focal areas of necrosis. Whereas Group T3 birds revealed comparatively normal liver parenchyma. The results of metabolism studies showed slow release of the parent compound from the body which might be the reason for mild restoration of toxic changes. Heart from Groups T2, T3 and T4 birds showed similar lesions of moderate intensity indicating restoration effect of withdrawal of both toxicants. Kidney sections suggested mild recovery of renal parenchyma in the form of mild to moderate granular and vacuolar changes in the tubular and glomerular epithelium but complete recovery was not recorded suggesting delayed toxicity effect even at 7 th day post withdrawal. Intestine from Groups T2 and T3 birds revealed mild to moderate degenerative changes indicating mild restoration with increase in cellularity. Group T4 birds exhibited moderate restoration. Brain from Group T2 and T3 birds showed mild neuronal degeneration and vacuolar changes in the cytoplasm. Whereas, Group T4 birds showed increase in number of glial cells, increased Virchow-Robin spaces and mild neuronal degeneration. Longitudinal sections of sciatic nerve from Groups T2, T3 and T4 showed mild degenerative changes compared to 4 th week indicating restoration towards normally. Previous studies revealed that chlorpyrifos and its principal metabolites are rapidly
eliminated from the body which might be the reason for restoration of lesions at 7th day post withdrawal. It had been reported by various workers that chlorpyrifos is rapidly absorbed in the body however its half life in body tissue varies from 62 h to 4 days (Gallo and Lawryk 1991). Also cypermethrin is readily excreted from the body and its half life has been recorded to the extent of 5 days (Furuzawa et al. 1981). Studies indicated that chlorpyrifos and cypermethrin does not persist in body tissues for longer duration and does not have a significant bioaccumulation which might be the reason for restoration recorded in various organs in toxicity groups after withdrawal of toxicants at 7th day post withdrawal. However, combined toxicity group showed mild degenerative lesions indicating mild to moderate restoration which might be due to delayed toxicity of combined effect of both. However, literature revealed lack of information on post withdrawal effect of either chlorpyrifos or cypermethrin or their combination in any of the species, hence need further exploration.

Ultra structural observations: At the end of 4th week, ultra structure of liver from control group showed clear chromatin, dense prominent nucleus with intact nuclear membrane, cytoplasm with vacuoles and mitochondria having clear cristae. Sections of liver from Group T2 birds showed lipid vacuoles and residues of cell organelles along with severe loss of cytoplasmic organelles. Mitochondria showed degenerative changes (Fig. 1). Present findings are in line with previous observation of Savithri et al. (2014). Ultra sections of liver from Group T3 birds showed eruption, perforation and swelling of nuclear wall, moderate to severe loss of chromatin material, elongation of Kupffer cells, mild dilatation of bile duct and vacuolated mitochondria. The sections of liver from Group T4 birds showed similar change as that of Group T2. Cytoplasm showed residues of cell organelles, destruction of microvilli, mitochondrial swelling, degeneration, more number of fat droplets and mild dilatation of bile duct (Fig. 2).

The changes in mitochondria were connected with disturbances in oxido-reduction processes taking place in the organelles and increase in peroxisomes may be associated with cellular response to the toxic effect of free radicals induced by chlorpyrifos (Savithri et al. 2014). The present findings of swollen mitochondria, dilatation of bile duct with destructed microvilli, elongated Kupffer cell and fat droplets are in accordance with previous observations in rat (Abdul-Hamid et al. 2017) regarding cypermethrin toxicity given @ 30 mg/kg/day. However, literature did not reveal ultra structural changes in liver intoxicated with chlorpyrifos and cypermethrin in combination.

The present findings thus clearly indicate that chlorpyrifos (@ 5 mg/kg feed) and cypermethrin (@ 200 mg/kg feed) given either individually or in combination cause pathological changes in cell organelles. The histoopathological lesions were more prominent in Group T4 given chlorpyrifos and cypermethrin in combination followed by Groups T2 and T3. The oxidative cytotoxic changes in hepatocytes were highly appreciated in ultra structure study of liver. Withdrawal of toxicants for 7 days could not completely restore the toxicopathology induced by chlorpyrifos and cypermethrin given either singly or in combination.

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REFERENCES


