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Patho-morphological effects in broiler birds induced with sub-acute chlorpyrifos toxicity and its amelioration with vitamin E and selenium

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Abstract

Day old broiler chicks (60) procured from a commercial hatchery were divided into 3 equal groups consisting of 20 each (T₁, T₂ and C) for the present study. About 1/10th of acute LD₅₀ i.e., 2 mg/kg body weight of chlorpyrifos was dissolved in 0.2 ml of coconut oil and fed orally with the help of a specially prepared syringe to each bird of treatment groups (T₁ & T₂) daily starting from day-old to 6 weeks or until death. Birds of T₂ group were also provided with selenium and vitamin E (E-care Se) @ 5gm/50 broiler birds through drinking water from day-old to 6 weeks or until death. Twenty birds of other group (C) received only the standard feed and plain water, serving as control. Birds of all the groups were sacrificed by cervical dislocation at the end of 6 weeks and gross pathological lesions were recorded immediately after slaughter. Tissue pieces of liver, kidney, lungs, spleen, intestine and bursa of Fabricius were preserved in 10% formalin solution immediately after necropsy and processed for paraffin sectioning (3-5 μ thick). Sections were stained with Mayer's haematoxylin and eosin for histopathological examinations. Sub-acute chlorpyrifos toxicity was observed to induce hyperemia and haemorrhages in most of the organs. Cytopathologically, there was toxic hepatitis and nephrosis, tubular nephritis, necrotic enteritis, myocarditis, pneumonic conditions and lymphocytolysis in spleen and bursa of Fabricius of chlorpyrifos induced birds (T₁). Lymphocytolysis in the lymphoid organs clearly indicated that chlorpyrifos had a cytotoxic effect leading to immunosuppression. The intensity of cytotoxic changes in the parenchymatous organs of chlorpyrifos + selenium and vitamin E treated birds (T₂) were mild in nature indicating a partial protective effect of selenium and vitamin E in chlorpyrifos intoxication.

Keywords: broiler birds, pathomorphology, chlorpyrifos, selenium, toxicity, vitamin E

Introduction

Pesticides are used most commonly in the crop field for more production of foods with the advent of green revolution. In veterinary practice some pesticides are most commonly used as acaricides or ectoparasiticidal to control the pests in case of animals and birds. Among the several organophosphorus insecticides, chlorpyrifos (O-O-diethyl-O-{3, 5, 6 trichloro-2-pyridyl}-phosphorothioate), a broad spectrum insecticide has gained popularity in veterinary medicine throughout the world. It is used as an insecticide for grain, cotton, field, fruit and vegetable crops as well as on lawns and ornamental plants because of its effectiveness against a variety of pests, mites, lice and flies affecting the livestock and poultry (Strickland *et al.*, 1970) [19]. General agricultural uses of chlorpyrifos pose a serious hazard to life (Kidd and James, 1991) [9]. Chlorpyrifos has wide application as an ectoparasiticide in domestic animals and as a premise insecticide (Blagburn and Lindsay, 2001) [6]. Unfortunately, the biological activity of chlorpyrifos is not limited just to insects but if used indiscriminately it is also toxic to mammals and other non-target organisms in which the cholinesterase enzyme plays a vital role. Low level of chronic exposure to agricultural chemicals may not have clinically recognizable symptoms but could produce subtle cumulative effects that eventually affect the health of the organism (Bebe and Panemanglore, 2003) [5].

Chlorpyrifos (CPF) is readily absorbed into the blood stream through the gastrointestinal tract if it is ingested, inhaled through the lungs or contact through the skin if there is dermal exposure (U.S. Public Health Service., 1995) [23]. The acute oral LD₅₀ of chlorpyrifos is 118-245 mg/kg for rats, 1000 mg/kg for rabbit, 504 mg/kg for guinea pigs and 10.79 mg/kg body weight for Chicks (USEPA, 2000) [22]. Acetylcholinesterase (AChE) inhibition and the induction of oxidative stress is one of the molecular mechanisms that have been implicated in CPF induced toxicity (Gultekin *et al.*, 2001 and Akande *et al.*, 2014) [7, 1]. Studies have shown that repeated exposure to CPF results in anemia, increased lipid peroxidation and oxidative stress as has been observed in the erythrocytes of rats following repeated CPF exposure

(Mansour and Mossa, 2009 and Ambali, 2009) [15, 3]. By-products of lipid peroxidation have been shown to cause profound alterations in structural organizations and functions of the cell membranes (Van Ginkel and Sevanian, 1994) [24]. To combat the menace of lipoperoxidative changes and oxidative stress, erythrocytes are endowed with arrays of endogenous antioxidant defense systems, including vitamins C and E, which act as potent scavengers of free radicals (FRs) (Kollanjiappan *et al.*, 2002) [10]. Vitamin E is the major lipid soluble antioxidant present in all cellular membranes where it protects the membranes against lipid peroxidation (Machlin, 1980) [14]. Vitamin E plays an important role in the maintenance of structural integrity of the erythrocytes protecting it from oxidative damage. Dietary vitamin E was found to improve reproduction and antioxidant capability of breeder chickens (Lin *et al.*, 2005) [13]. Selenium is widely distributed in animal tissues in very small amount and is an important antioxidant that protects the body against free radicals and other oxidative damage (Mishra, 2007) [16]. The selenium compounds exert their biological effects either directly or by being incorporated into enzymes and other bio-active proteins. Accidental or careless applications of organophosphorus compound have resulted in the death of many species of non target organisms such as fish, aquatic invertebrate, birds and humans. Due to presence of residue of chlorpyrifos in the soil, water, forages of ecosystem there is possibility of health hazards to the farm animals, poultry, human beings and wild animals. Till date few researches has been carried in India regarding the hazards and control of chlorpyrifos in poultry. Hence, the present research work was carried out to observe the patho-morphological effects in broiler birds induced with sub-acute chlorpyrifos toxicity and also to record the ameliorating effect of vitamin E and selenium in chlorpyrifos intoxicated broiler birds.

Materials and Methods

Day old broiler chicks (60) weighing 40-50 gm were obtained from a commercial hatchery. They were divided into 3 equal groups (T₁, T₂ & C) of 20 each to determine the toxicopathological effects of induced chlorpyrifos toxicity and its alleviation with vitamin E and selenium. Birds were housed in standard hygienic and managerial condition throughout the experimental period. All the birds were fed with a commercial chick starter ration containing 22% protein and 3000 Kcal ME/Kg up to 3 weeks, followed by a finisher ration having 19% protein and 2900 Kcal ME/Kg, formulated to meet nutrient requirement as recommended by NRC (1984) [17]. Feed and water were provided *ad libitum* throughout the study.

Technical grade of Chlorpyrifos (Chlorpyrifos technical^R, purity 98% w/w) was procured from Gharda Chemicals Limited, Ratnagiri, Maharashtra (India) and was used in this study. Vitamin E and Selenium (E-care Se) manufactured by Vetcare division of Provimi Animal Nutrition India Pvt. Ltd. Pharmaceutical company were utilized for the study.

Chlorpyrifos @2mg/ kg body weight i.e. about 1/5th of acute LD₅₀ of Chlorpyrifos (Al-Badrany and Mohammad, 2007) [2] fed orally using coconut oil as vehicle with the help of a specially prepared syringe to each birds of treatment groups (T₁ & T₂) consisting of 20 birds each daily starting from day-old to 6 weeks or until death. Birds of T₂ group was also provided with vitamin E and selenium (E-care Se) @ 5gm/50 broiler birds through water from day-old to 6 weeks or until death. Twenty birds of other group (C) were provided standard feed and plane water only, serving as control. All the

experimental birds (T₁, T₂ & C) were observed daily for the development of clinical signs of toxicity including mortality (if any). Birds of all the groups were sacrificed by cervical dislocation at the end of 6 weeks and gross pathological lesions were recorded immediately after slaughter. Tissue pieces of liver, kidney, lungs, spleen, intestine and bursa of Fabricius were preserved in 10% formalin solution immediately after necropsy and processed for paraffin sectioning (3-5 μ thick). Sections were stained with Mayer's haematoxylin and eosin for histopathological examinations (Bancroft and Stevens, 1980) [4].

Results and Discussion

Grossly, CPF treated birds (T₁) showed pale discoloration and enlargement of liver with severe haemorrhages (Fig.1). Both the kidneys were slightly swollen with varying degree of congestion and hemorrhages (Fig.2). The spleen and bursa of Fabricius of intoxicated birds were also swollen and hyperemic (Fig.2). The small intestine showed thickening of the wall and was hyperemic. The lumen was covered with slimy, stringy and viscid mucous. The intestine also revealed mild haemorrhages (Fig.3). Both the lungs were congested and at places, showed focal areas of consolidation. The birds treated with CPF + selenium and vitamin E revealed pale discoloration of liver and mild congestion on the surface (Fig.4). Spleen of the birds of these groups had also mild congestion. The birds of the CPF + selenium and vitamin E treated groups at necropsy showed mild congestion and haemorrhages in the kidney, and the lungs of the birds of this group had only mild congestion indicating partial ameliorative effect of selenium and vitamin E. The intestine of CPF + selenium and vitamin E group also had mild changes consisting of mild hyperemia and catarrh in the lumen (Fig.4). Kammon *et al.* (2010) [8] observed similar changes in the parenchymatous organs of chicken induced with CPF toxicity. Sodhi *et al.* (2008) [18] also reported the similar changes in the different parenchymatous organs of chicks induced with CPF toxicity and vitamin E ameliorated birds.

Histopathological lesions consisted of moderate to severe congestion, hyperemia, cellular swelling with granular cytoplasm, sinusoidal dilatation, degeneration and coagulative necrosis of hepatocytes in the centrilobular and peripheral areas of liver in the birds of T₁ group (Fig. 5). Some of the liver section showed mononuclear cell infiltration. These lesions were of less intensity in CPF + vitamin E and selenium treated birds (Fig. 6.). Similar lesions in the liver were reported by Tripathi and Srivastava (2010) [21] in chlorpyrifos treated birds, by Sodhi *et al.* (2008) [18] in both chlorpyrifos treated birds and chlorpyrifos + vitamin E and selenium treated broiler chicks. Oxidative stress induced by CPF might be cause of cellular swelling and hepatic degeneration. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells.

Microscopically, congestion and haemorrhages in the kidney and condensation of nuclei of the tubular epithelium, degeneration and necrosis of the tubular epithelium (Fig.7) in CPF treated birds were observed. Only mild degenerative or cytotoxic changes (Fig.8) were found in the kidney of CPF + vitamin E and selenium treated birds. The histopathological findings of kidney in birds of all the groups of the present study corroborated with the findings of Kammon *et al.* (2010) [8] in layer chickens and Kumar (2011) [12] in broiler birds.

Different sections of spleen showed severe congestion, haemorrhages, necrosis and depletion of the lymphoid cells in

the Malpighian corpuscles of white pulp (Fig.9) in the CPF treated birds. The spleen of the birds of the ameliorative groups revealed only mild to moderate congestion and mild depletion of the lymphoid cells in the spleen (Fig.10) indicating partial protective effect of vitamin E and selenium in sub-acute CPF poisoning of broiler birds. Similar type of cytotoxic changes had also been reported in spleen of the broiler birds intoxicated with chlorpyrifos for 28 days (Krishnamoorthy *et al.* 2007)^[11] and in spleen of Wistar rats exposed to glyphosate (Tizhe *et al.*, 2014)^[20].

The intestine of CPF treated birds (T₁) showed degeneration and desquamation of lining epithelial cells, necrosis of the villi, and sloughing off in the lumen of the intestine with heterophil and mononuclear cells infiltration. Some of the sections of the intestine of the birds of the T₁ group also showed haemorrhages in the submucosal layer, necrosis and denudation of the villi in the lumen of the intestine. Birds of T₂ showed only mild thickening of lining epithelial cells in the submucosal layer indicating protective effects of vitamin E and selenium on the intestinal damage caused by CPF. The changes found in the intestine of chlorpyrifos treated birds in the present study simulated the reports of the Kumar (2011)^[12] in birds induced with chlorpyrifos. Histopathologically, the lungs of the birds of T₁ group revealed marked congestion of alveolar capillary with or without extravasation of erythrocyte (Fig. 11). Some of the sections had light pink colour oedematous fluid. In few sections of lungs, there was infiltration of heterophils and mononuclear cells. The lungs of the birds of group T₂ revealed mild to moderate congestion only (Fig. 12) indicating the protective effects of vitamin E and selenium. The changes found in the lungs of chlorpyrifos treated birds in the present study simulated the reports of the Kumar (2011)^[12] in broiler birds. The heart of the birds of T₁ group revealed mild congestion and degenerative changes in myofibers with or without extravasation of erythrocytes in between the myofibers (Fig. 13). On the other hand, the birds of T₂ group had only mild degenerative changes in myofibers of heart (Fig. 14). The histopathological changes found in the heart of chlorpyrifos treated birds in the present study corroborated the reports of the Kumar (2011)^[12] in broiler birds. No literature is available to compare the effect of vitamin E and selenium in the lungs, intestine and in the heart of chlorpyrifos induced broiler birds.

Histopathologically, the bursa of Fabricius of the birds of T₁ group showed congestion, mild to severe lymphocytolysis and depletion of lymphocytes in bursal follicles and degeneration and desquamation of lining epithelium (Fig. 15). Similar microscopic lesions were observed in the birds of group T₂ but the changes were mild in nature (Fig. 16). Almost same type of cytotoxic changes had also been reported by Krishnamoorthy *et al.* (2007)^[11] in spleen of the broiler birds intoxicated with chlorpyrifos for 28 days.

The birds of T₂ group fed with CPF @ 2mg / kg b.wt. with supplementation of vitamin E and selenium revealed mild pathomorphological lesions as compared to the birds of group T₁ indicated biological protective role of vitamin E and selenium. Antioxidants have long been attributed to be the reducers of the free radical-mediated oxidative stress. The defensive role of selenium and tocopherol in biological tissue damage has been reported in chicks by Sodhi *et al.*, (2008)^[18]. It is also interesting to note that the lesions of pneumonia and myocarditis which were observed in some of the birds of T₁ group but not observed in vitamin E and selenium treated group. The ameliorative effect of selenium and vitamin E appears to play an important role in regulating redox potential

through GPx, thioredoxin reductase and selenoproteins in scavenging hydroperoxide generated by OP compounds (Machlin, 1980)^[14]. GPx and thioredoxin reductase are to natural antioxidant enzymes that contain selenium and depend upon selenium activity for their antioxidant function thereby minimizing tissue damage. The possible molecular mechanism of tissue toxicity caused by chlorpyrifos is by generation of reactive oxygen species causing membrane damage of cell resulting into degeneration and necrotic changes in various organs. Presumably, it is possible that CPF caused severe toxic injury to capillary endothelium resulting to the development of widespread vascular lesions in various organs. However, necrosis and depletion of lymphoid cells in the immunobiological organs like spleen and bursa of Fabricius clearly suggest that chlorpyrifos has a cytotoxic effect, so that immunosuppression may be a possible outcome of sub-acute chlorpyrifos intoxication.



Fig 1: Enlargement of Liver with hemorrhages in the birds of T₁ group.



Fig 2: Enlargement and hemorrhages in the kidney and Swollen spleen and bursa of Fabricius in the birds of T₁ group.



Fig 3: The lumen of intestine (hemorrhagic) covered with slimy, stringy and viscid mucous in the birds of T₁ group.



Fig 4: Mild hyperemia and catarrh in the lumen of intestine of T₂ group birds

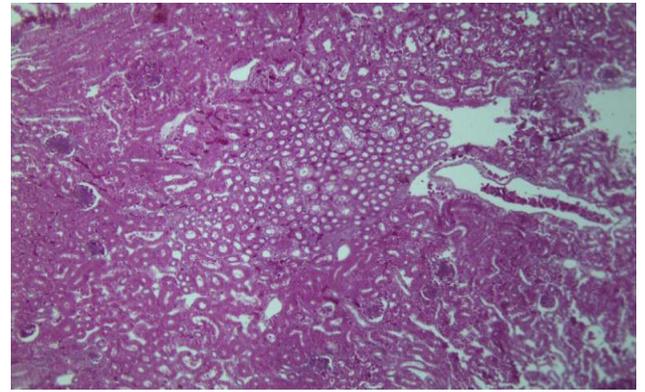


Fig 8: Mild congestion and degeneration of the tubular epithelium (T₂). H&E X 100

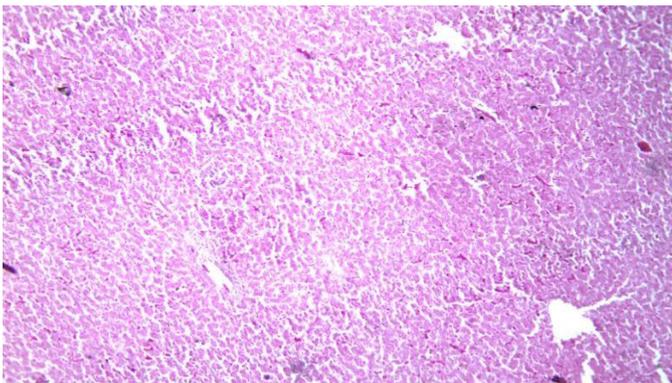


Fig 5: Sinusoidal dilatation, degeneration and coagulative necrosis of hepatocytes (T₁). H&E X 100

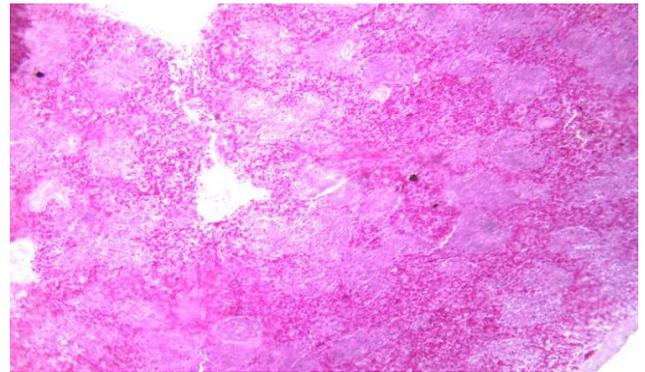


Fig 9: Haemorrhages and severe depletion of the lymphoid cells in spleen (T₁). H&E X 100

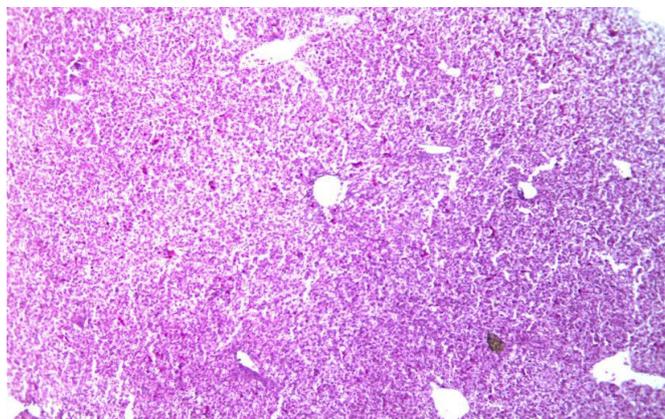


Fig 6: Mild cytotoxic changes in liver (T₂). H&E X 100

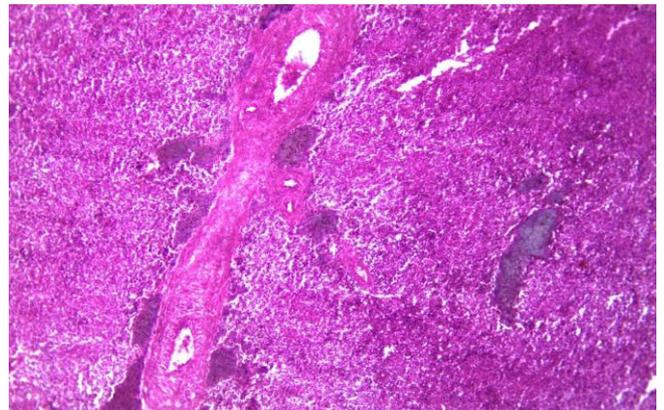


Fig 10: Congestion and mild depletion of the lymphoid cells in spleen (T₂). H&E X 100

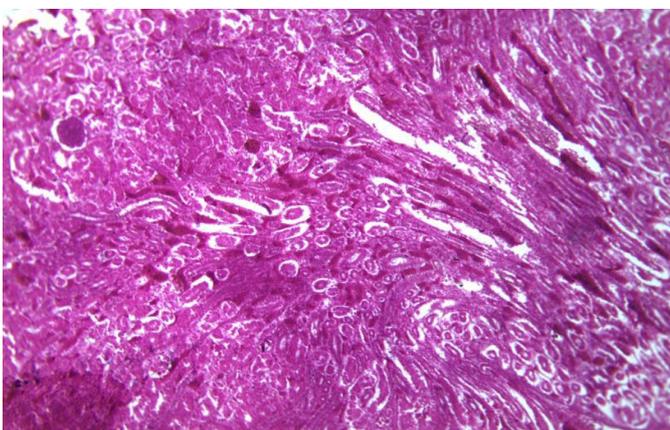


Fig 7: Congestion, degeneration and necrosis of the tubular epithelium (T₁). H&E X 100

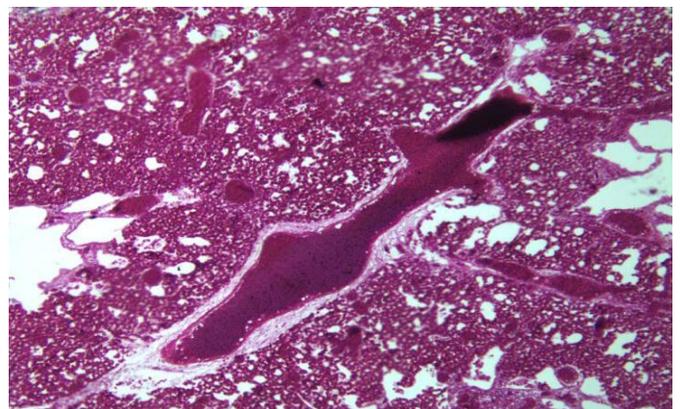


Fig 11: Marked congestion of alveolar capillary of lung (T₁). H&E X 100

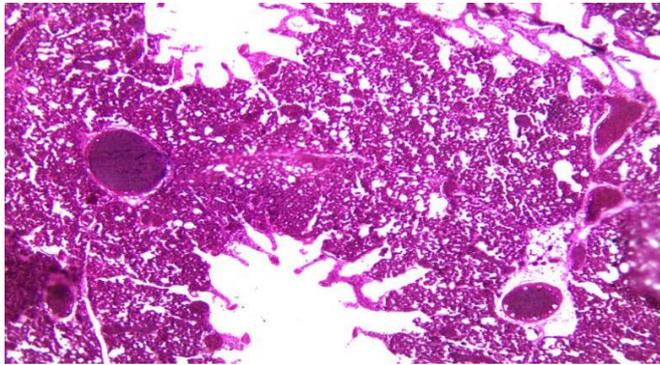


Fig 12: Mild to moderate congestion of alveolar capillary of lung (T₂). H&E X 100

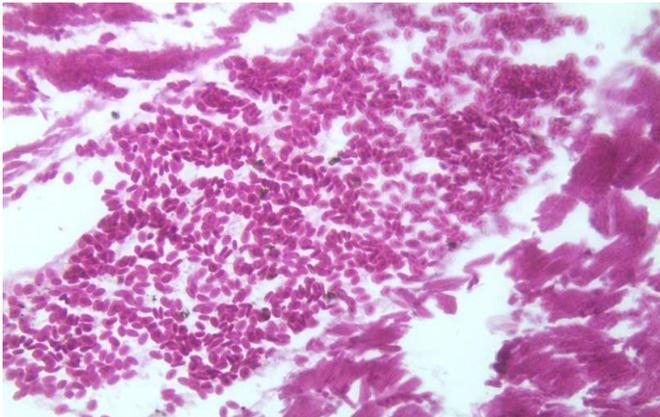


Fig 13: Degenerative changes in myofibers with extravasation of erythrocytes in between the myofibers (T₁). H&E X 400

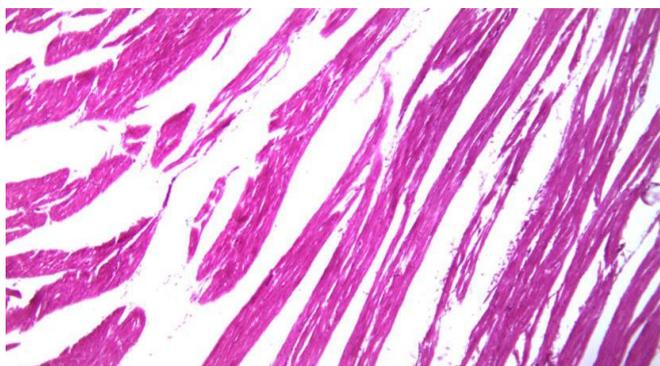


Fig 14: Mild degenerative changes in myofibers of heart (T₂). H&E X 150

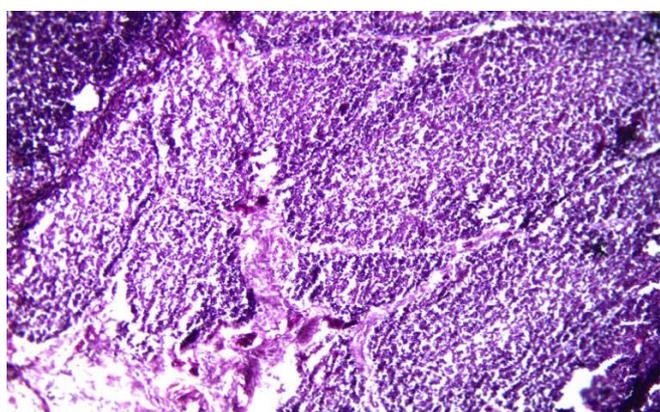


Fig 15: Severe congestion, Lymphocytolysis, degeneration and desquamation of lining epithelium & depletion of lymphocytes in bursal follicles (T₁). H&E X 200

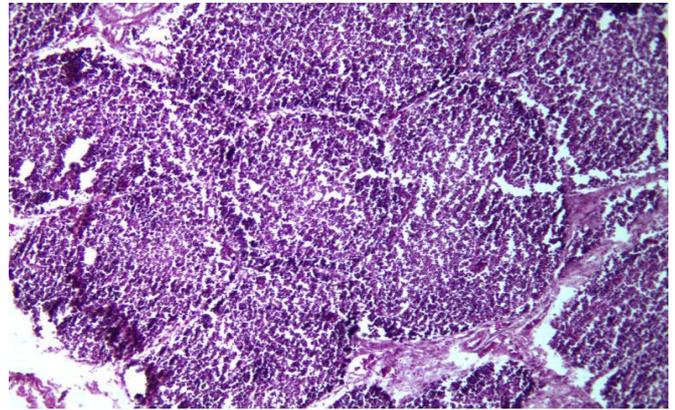


Fig 16: Mild congestion & depletion of lymphocytes in bursal follicles (T₂). H&E X 200

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