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Dr. Majid Shafi Kawoosa

Assistant Professor, Faculty of Veterinary Sciences and Animal Husbandry, Division of Veterinary Pathology, Government Veterinary College, Mhow, Madhya Pradesh, India

Shabia Khan

Junior Scientist, Faculty of Veterinary Sciences and Animal Husbandry, Division of Veterinary Pathology, Government Veterinary College, Mhow, Madhya Pradesh, India

Pathomorphological studies of Nimesulide induced sub-acute toxicity in Japanese quails

Dr. Majid Shafi Kawoosa and Shabia Khan

Abstract

This experimental work was conducted to study the pathomorphological changes of Nimesulide induced toxicity in Japanese Quails. A total number of 100 healthy Japanese quails were reared up to two weeks of age and then divided into four major groups i.e. Control (C) group and the three Treatment groups namely N1, N2 and N3 respectively. Experiment was carried out on 100 birds out of which 25 birds were kept in each group. The birds in control group received normal starter feed without any medicine and the drug was given with feed @ 10, 20, and 30 mg /kg body weight to the birds of N1, N2 and N3 groups for 15 days. The intoxicated birds revealed nephropathy and depletion of lymphoid tissue from the lymphoid organ which were suggestive of immunosuppressive effect of Nimesulide in intoxicated birds.

Keywords: Nimesulide, toxicity, nephropathy, lymphoid depletion and immunosuppression

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the class of drugs that reduce pain, decrease fever and prevent blood clots. Nonsteroidal anti-inflammatory drugs have the ability to inhibit cyclooxygenase enzymes, which are involved in the formation of prostaglandins. There are at least three variants of cyclooxygenase enzymes namely COX-1, COX-2 and COX-3. Nimesulide is non-steroidal anti-inflammatory drug first launched in Italy in 1985. Nimesulide is believed to inhibit COX-2 enzyme in order to produce analgesic, antipyretic and anti-inflammatory effects, but continuous use of these agents increases the risk of toxicity in animals and birds. In addition to cyclooxygenase enzyme inhibition and reduced prostaglandin synthesis, it also reduces generation of superoxide by neutrophils, decreases histamine release from tissue mast cells and blocks metalloproteinase activity. Nimesulide is held responsible for the topical toxicity at high doses and is associated with inflammation and ulceration. Nimesulide induced liver injury gained investigative interest and was found to produce hepatocellular necrosis with adverse gastrointestinal effects which fatal in nature. Nimesulide was markedly toxic to birds, resulting in severe clinical toxicosis and even death following either acute intoxication with high doses or slow intoxication with repeated exposures, thus presenting this Nonsteroidal anti-inflammatory drugs as an important environmental contaminant and health hazard in birds. This is especially important in view of the decline of bird populations recorded over past few decades.

Keeping the above facts in view, this investigation was designed to study the Pathomorphological changes of Nimesulide Induced Sub-Acute Toxicity in Japanese quails

Materials and Methods

Experimental birds: A total number of 100 one-day old Japanese quails were procured from the local market of Indore situated in the vicinity of the Faculty of Veterinary Sciences. The birds were already vaccinated against Marek's disease prior to the delivery. On the first day of procurement the birds were given electrol in water in order to get rid dehydration and transportation stress.

Housing: The room was thoroughly cleaned and every part of the room was flamed. Next day white washing of the room was carried out prior to housing of the birds, the room was further fumigated with a potassium permanganate and formalin. The birds were housed in the deep litter system and a floor space of 2 square feet was provided to each bird. Each group had a separate marking with wire mesh. Thoroughly dried and disinfected chopped paddy straw was used as bedding for the birds.

Corresponding Author:

Dr. Majid Shafi Kawoosa

Assistant Professor, Faculty of Veterinary Sciences and Animal Husbandry, Division of Veterinary Pathology, Government Veterinary College, Mhow, Madhya Pradesh, India

Grouping: Experiment was carried out on 100 birds out of which 25 birds were kept in each group. The birds were divided into four major groups i.e. Control (C) group and the three Treatment groups namely N1, N2 and N3 respectively.

Feeding: All the birds irrespectively of their groups were maintained on the same type of starter mash. The birds were fed in the first week @ 10g / bird but from second week onwards ad.lib. feeding was adopted. The birds of each group has a separate feeding and watering trays.

Vaccination: All the birds were vaccinated against Marek's disease on the first day of hatching whereas F1 (Lasota) vaccination against Ranikhet disease was carried out to all birds on 6th day of age.

Drug Dose: The dose was selected on the basis of LD-50 estimated in the Pretrial conducted at the Divisional level. The LD50 in the Japanese quails was calculated @ 30 mg /kg body weight. The birds in control group received normal starter feed without any medicine and the drug was given with feed @ 10, 20, and 30 mg /kg body weight to the birds of other groups for 15 days.

Technical programme: Experiment was carried out on 100 birds out of which 25 birds were kept in each group. The birds were housed in the deep litter system and dried paddy straw was used as bedding material for the birds. The birds were reared up to 2 weeks of age and then divided into 2 major groups i.e. control (C) group and the treatment (T) group. The Treatment group (T) was further divided into 3 sub-groups namely N1, N2 and N3 respectively. The control birds received normal starter feed without any medicine and the Ibuprofen was given with feed @ 10, 20 and 30 mg /kg body weight to the birds of N1, N2 and N3 groups for a period of 15 days. Each bird was critically inspected every day for the appearance of clinical symptoms of toxicity at every stage of the experiment. The mortality rate in each group was also recorded every day until the end of the experiment which lasted for 30 days.

Gross pathology: All the surviving experimental birds were sacrificed and each organ was critically examined for macroscopic lesions. The lesions were recorded and compared at the end of the experiment.

Histopathology: All the dead and sacrificed birds were subjected to necropsy examination. At necropsy table, pieces of different lymphoid organs like spleen, bursa, caecal tonsils and thymus were collected and fixed in 10% buffered formalin for histopathological examination. Later, paraffin embedded tissue sections were cut at 5 µm thickness and stained with hematoxylin and eosin (Lillie, 1954) ^[4] in order to observe pathomorphological changes in the lymphoid organs.

Experimental findings

Clinical Signs: The intoxicated birds of NI group were dull and depressed followed by anorexia. About half an hour after intoxication, the birds layed down and lacrimation was observed. The affected birds stood motionless with both eyes closed. There was hyper salivation with decreased respiratory rate, pupil was dilated and birds showed a staggering gait. Also, the intoxicated birds vomited suddenly and passed

undigested food in faeces. Three out of 25 birds died due to Nimesulide intoxication giving mortality rate of 30 percent during the study period. The marked clinical signs observed in intoxicated birds included huddling, dullness, appeared listless, depressed and showed laboured breathing. The birds were reluctant to move and stood with ruffled feathers and cyanosed combs. Some of the affected birds also revealed lameness, enlarged hock joints and sternal recumbency. After 6 hours, birds exhibited increased thirst, droopy wings, and diarrhoea. Birds that died rapidly showed pronounced neurological signs like convulsions. The mucus membranes of the affected birds were pale and emaciated. The birds which died fell down suddenly and death occurred within one hour due to respiratory failure.

The intoxicated birds of N2 group appeared weak, depressed and succumbed within 5 days after intoxication. There was loss of feather and excessive salivation. The affected birds exhibited growth retardation, twisted necks and lameness. The emaciation and dehydration was noted in the second week of study period. The clinical signs were more evident in birds after 10 days of post intoxication. The intoxicated birds revealed depression, drowsiness, inappetence, huddling together, drooping of wings, dehydration, laboured breathing, lowering of head, ruffiness of feathers and weakness. Diarrhoea was apparent 5 to 6 days after intoxication and the droppings were yellow in colour. At day 10, the respiratory movements were decreased and birds appeared to have difficulty in breathing. These intoxicated birds appeared ruffled with pale head and shrunken comb. There was a decline in body weight during the second week of intoxication. A total number of five birds died and all the birds died between 15 days of post intoxication. The clinical signs observed in the second week of intoxication included tibial dyschondroplasia, lameness, abnormal bending of the tibial bones, enlarged hock joints, sternal recumbency convulsion, lacrimation from eyes, rough feather coat, profuse salivation, diarrhoea, restlessness and ataxia. The keel bone appeared sharp due to emaciation. Other major clinical signs observed in intoxicated birds were yellowish diarrhoea, emaciation and polydipsia. The intoxicated birds showed significant increase in temperature beginning from the 1st day of intoxication till end of the study period. At the end of the experimental period, the intoxicated birds which survived for few days revealed retarded growth, hemorrhagic enteritis, watery droppings, muscle weakness, staggering gait and loss of response to stimulus. The intoxicated birds stood motionless for long period of time with the feathers puffed and both eyes closed. The birds showed repeated sneezing and fluid came from mouth which appeared to be clogged.

The affected birds of N3 group exhibited anorexia, dyspnea, apathy, lethargy, staggering gait, and death. After 10 days of intoxication, there was decrease in feeding, polydipsia, severe depression, listlessness, reluctance to move, standing with sunken head, and greenish diarrhoea. A total number of 20 birds succumbed to intoxication during the experimental period. The onset of the symptoms was recorded on 10th day and reached its peak at the end of experimental period in intoxicated birds. There was a sudden drop in feed consumption and the birds appeared droopy, ruffled feathers with pale head and shrunken comb. Marked clinical signs appeared on 15th day when half of the intoxicated birds showed pasted vent, laboured breathing and distorted body appearance. The intoxicated birds showed emaciation, depression, lameness and sternal recumbency. Birds layed

down with their feathers fluffed out for prolonged periods of time. Also, the intoxicated birds showed the altered behavior such as reduced attentiveness, restlessness and hyper salivation. At later stage nervous signs were marked, which included ataxia, convulsions, dilation of pupil and respiratory depression. At the end of experimental period, the important clinical signs observed were dullness with closed eyes, loss of appetite, drowsiness and watery diarrhoea leading to death. Death usually resulted from respiratory failure. Before death, the birds fell suddenly revealing paddling of legs and unconsciousness.

Pathomorphological Changes

Gross Pathology

Liver: The most predominant gross lesions observed in liver were yellowish discoloration which was accompanied with petechial hemorrhages in many cases. Frequently, liver was enlarged and congested which were consistently observed throughout the study period. On palpation, livers appeared firm and large irregular necrotic foci were also noted on its surface in most of the intoxicated birds. At early stage liver appeared normal and at later stage it was darker, enlarged, swollen, congested and frequently necrotic areas were found on the liver surface. Postmortem examination of the birds after one week of drug intoxication revealed yellowish discoloration, Peel off the liver capsule and cooked appearance. The liver of intoxicated birds revealed marked enlargement and hemorrhage in early stages whereas necrosis and hypertrophy were observed at the last stage of experimental period.

Kidneys: Kidneys were generally appeared enlarged, rough and congested. Varying degrees of haemorrhages, predominantly ecchymotic were also evident on surface of the kidneys. Kidneys were apparently normal on 5th day whereas at day 8 onwards, kidneys were congested with grayish white necrotic foci present on the surface of the kidneys. After one week of intoxication, the kidneys of the affected birds were generally rough, necrotic, congested and revealed ecchymotic haemorrhages on the surface. Nephropathic changes were more severely observed in the kidneys of the experimental birds at the end of study period. The prolonged exposure of drug also resulted in a significant increase in the weights of kidneys from day 12 onwards when compared with the weight of kidney of control birds. Post-mortem examination of the intoxicated birds at the end of second week revealed mottled kidneys with necrotic foci present on the surface.

Lungs: Lungs appeared emphysematous, Congested and ecchymotic haemorrhages were predominantly observed in the right lung while left lung in some cases revealed suffusions and areas of red hepatization. The birds sacrificed at the end of first week revealed varying degree of consolidation along with congestion. In few cases on 10th day grayish area of consolidation were noted on the right lung which on incision revealed presence of creamy viscous exudate oozing out from the lung surface. At the end of the experimental period, the right lung of intoxicated birds were generally emphysematous and revealed ecchymotic haemorrhages whereas left lung in few cases revealed areas of congestion and consolidation.

Heart: Heart revealed gelatinization of pericardium and the quantity of pericardial fluid was found to be increased in

intoxicated birds which were mainly observed in the first week of study period. In some cases petechial haemorrhages were also noted on the pericardium of the intoxicated birds after the oral administration of the drug. The intoxicated birds at the end of first week revealed pericarditis and gelatinization of the fat associated with the pericardium. Postmortem examination of the intoxicated birds at the end of the experimental period revealed enlargement of the heart, ecchymotic hemorrhages and white necrotic foci on the surface.

Proventriculus: Proventriculus generally appeared enlarged, edematous and congested with serous exudate present in the lumen throughout the experimental period. Varying degree of haemorrhages were also observed on the mucosal surface of the proventriculus after the oral administration of drug. Post mortem finding revealed an elevated borders of the proventriculus which were most frequently seen in advanced cases at the end of study period. The intoxicated birds on post mortem revealed multifocal ulcerations on the mucosa of the periventricular and thinning of the wall of proventriculus. Upon opening of the proventriculus of the intoxicated birds which were succumbed during the experimental period revealed liquid mucus which covers the mucosa of the proventriculus. In advanced cases, the mucus became whitish and thick which were easily visualised with the naked eye. The wall at the transitional area from the proventriculus to the gizzard appeared weakened. In severe cases, the Coilin layer of the proventriculus became spontaneously loosened and detached from the mucosa.

Gizzard: The Gizzard of intoxicated birds showed hemorrhages and necrotic spots on the surface of the organ throughout the study period. Macroscopically, discoloration and erosion of the Koilin layer of the gizzard were observed in all intoxicated birds. Postmortem examination of intoxicated birds succumbed during the experimental period revealed inflammation along with petechial haemorrhages of the mucosa of the gizzard. The birds sacrificed at the end of experimental period revealed ulcerative mucosa and myopathy of the Gizzard which was characterized by formation of grey areas in the gizzard muscle. The affected gizzard muscle of the intoxicated birds are firmer in texture as compared to the gizzard muscle of control birds. The intoxicated birds also revealed significant changes in the gizzard which may include cracks, shows furrows and upon peeling off dry bloody ulcers which were mostly observed at the end of study period.

Brain: Involvement of the brain appears to be secondary to massive hepatic necrosis. The gross lesions of the nervous system comprised of congestions in the brain, oedema and encephalitis which were seen more severe in the succumbed birds at the end of experimental period. After the intoxication of one week, the brain of intoxicated birds revealed the gross lesions of hardened and circular necrotic areas on the cerebrum. Birds sacrificed after day 10 revealed hemorrhagic lesions, pale discoloration, oedema and necrotic spots on the surface of brain.

Crop: Grossly, the crops of the intoxicated birds were distended and gelatinized after the first week of intoxication. Post mortem finding of the intoxicated birds revealed viscous mucinous fluid in the lumen of crop throughout the study

period. The birds sacrificed at the end of experiment revealed oedema and thickening of the wall of the crop.

Joints: The birds intoxicated with Nimesulide revealed swollen hock and toe joints. After one week of intoxication, there was deposition of viscid material in joints resulting in the lameness of the birds. Post mortem examination of the intoxicated birds after second week, revealed viscid thick mass of urates like substance observed in most of the joints and over the tendon sheath. Deposits were also observed in almost all other joints including shoulder, elbow, carpus and phalanges in wings and hip, knee and toe joints in the limbs. Rarely some deposits were also observed in the cervical articulations. At the end of experimental period, the post mortem of intoxicated birds reveals epiphyseal abnormalities in the long bones as well as severe enlargement of hock and phalangeal joints.

Spleen: The lesions in intoxicated birds were characterized by enlargement and pinpoint necrotic foci found on the surface of spleen. Hemorrhages were also observed on the surface of spleen of intoxicated birds after 48 hours of oral administration of drug. The spleen showed marked enlargement and congestion in early stages of experimental period where as necrotic patches were also observed uniformly distributed on their surfaces at later stages of study period. After 10 days of intoxication, the spleen of affected birds appeared mottled with grayish necrotic spots on the surface of the organ. Vascular congestion and hemorrhages were consistently observed on the surface of the spleen throughout the study period. At the end of the experimental period, spleen appeared to be atrophied with rough surface. The atrophy of spleen might be due to the depletion of lymphoid tissue from the lymphoid organ.

Bursa: Hemorrhages were more prominent in bursa of Fabricius of intoxicated birds which was easily visualized on gross examination. At early stage of experimental period, there was deposition of viscid material in the bursa of Fabricius along with the discoloration of the organ. Necrotic spots were frequently noted in bursa of Fabricius which was also atrophied after day 10 due to the depletion of the lymphoid tissue. Postmortem examination at the end of experimental period revealed moderate vascular congestion, reduced in size of organ and interstitial edema.

Thymus: Thymus of Nimesulide intoxicated birds appeared to be reduced in size due to the lymphoid depletion which was observed in the first week of the experimental period. Thereafter varying degrees of congestion and edema were consistently observed in the experimental birds. The thymus of certain intoxicated birds exhibited discoloration and necrotic changes which were observed after day 10 of post intoxication up to the end of study period.

Microscopic Lesions

Liver: Microscopically the liver of the intoxicated birds generally revealed sinusoidal congestion and varying degrees of hepatocellular degeneration (Fig: 1). Diffuse areas of necrosis characterized by Pyknosis and karyolysis of nucleus in hepatocytes associated with infiltration of lymphocyte and macrophages were observed in the experimental birds. The liver of the intoxicated birds at the end of first week of study period revealed hepatocellular swelling and the areas of

coagulated necrosis. In some cases hepatitis with mononuclear cells infiltration was also observed in the glisson capsule and portal areas. The epithelial lining of the bile ducts and canaculi of the nimesulide intoxicated birds revealed necrosis and cytolysis. At the end of the experimental period the livers from the intoxicated birds revealed moderate degenerative changes which was characterized by cellular swelling, focal hepatitis, dilatation of sinusoids and necrosis. The liver of the birds intoxicated with the nimesulide drug also revealed cytoplasmic eosinophilia, vacuolar changes, fibroplasia and thickening of capsules of the liver.

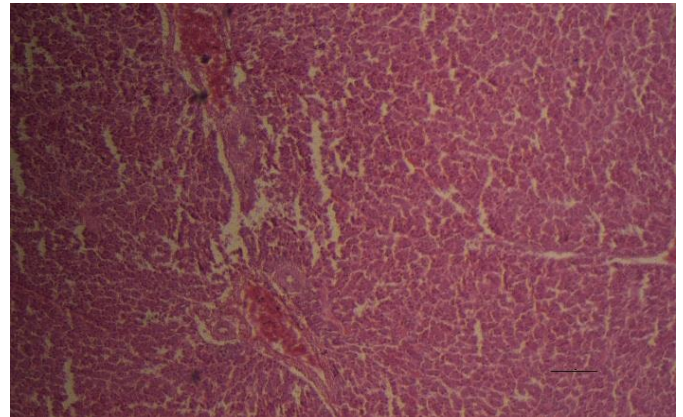


Fig 1: Photomicrograph of liver showing congestion and hepatocellular degeneration

Kidneys: On microscopic examination, kidneys revealed vascular congestion and degeneration with loss of tubular epithelium (Fig: 2). Renal tubules in the medullary region revealed degenerative changes characterized by swollen epithelium, irregular cell boundaries and increased eosinophilia. Nephritic changes characterized by cellular swelling and necrosis of epithelial lining in the convoluted tubules and ducts were more severe in birds died during the study period. Glomeruli of the intoxicated birds revealed hypercellularity, hypersegmentation and hyperplasia of meningeal cells. The birds died during the experimental period also revealed fibroplasia, cellular swelling and vacuolar changes in the epithelial lining of collecting tubules. At the end of study period, there were interstitial nephritis, denudation of epithelium lining and Hydropic degeneration with accumulation of eosinophilic hyaline mass in the lumen of tubules. Renal tissue also revealed deposition of tophi appearing as pink colour radiating structure in the Bowman's capsule, tubules and interstitial tissue.

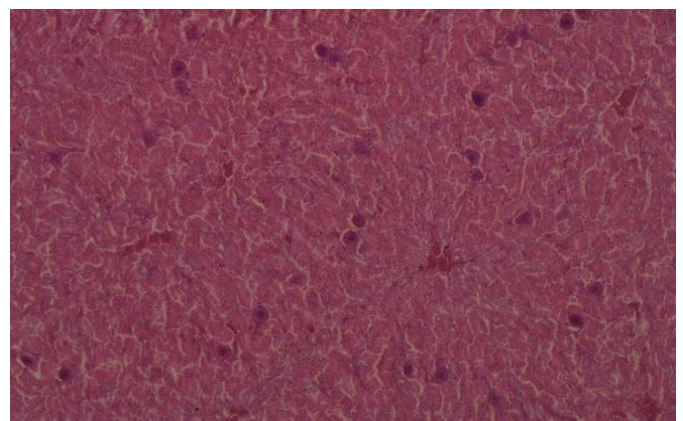


Fig 2: Photomicrograph of kidney revealed vascular congestion with loss of tubular epithelium

Lungs: The lungs of the intoxicated birds revealed severe congestion, hemorrhages and necrosis (Fig: 3). In most of the cases air vesicles were filled with edematous fluid and the walls of the air vesicles are thickened as compared to normal. Birds succumbing during the study period frequently revealed emphysema and the focal areas of bronchopneumonia characterized by the presence of necrotic tissue with cellular infiltration particularly lymphocytes in air vesicles, bronchi and bronchioles. At the end of experimental period, microscopic examination of the lungs of intoxicated birds also revealed edema and necrotic changes along with non suppurative pneumonia characterized by the infiltration of mononuclear cells in the parabronchi and secondary bronchi.

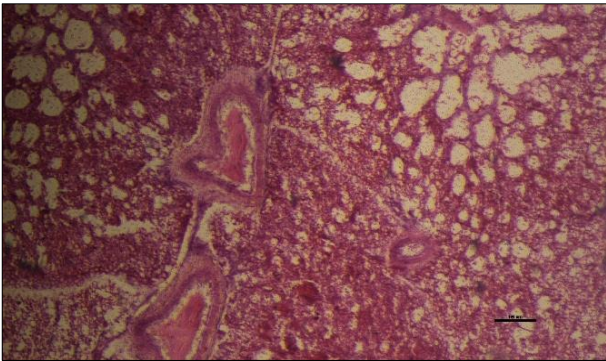


Fig 3: Photomicrograph of lung revealed hemorrhages and necrosis

Heart: Microscopically, the heart of the intoxicated birds revealed vascular congestion in pericardium and degeneration with separation of myofibers. Most of the cases revealed Zenker's degeneration of heart muscles, thickening of epicardium and myocardium characterized by infiltration of mononuclear cells. After first week of intoxication, the heart of intoxicated birds revealed disruption of heart muscles, vascular congestion in epicardium and marked degeneration of myocardium (Fig: 4). The birds succumbed during the study period revealed degeneration of myocytes characterized by cytoplasmic rarefaction giving vacuolar appearance in association with mononuclear cellular infiltration. The birds scarified at the end of experimental period revealed vascular congestion, focal hemorrhages and the degenerative changes characterized by cytoplasmic eosinophilia, nuclear pyknosis and nuclear rarefaction.

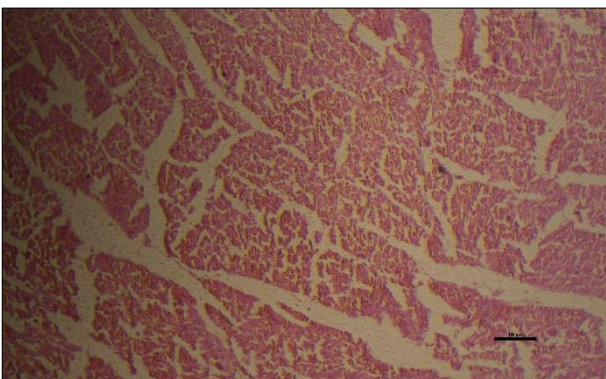


Fig 4: Photomicrograph of heart revealed disruption of heart muscles

Proventriculus: Vascular congestion, denudation and oedema of sub mucosa were a consistent feature throughout the study period. From first week onwards, Proventriculus showed hyperplasia of mucosa with heavy infiltration of

lymphocytes. The glandular epithelium of the proventriculus of intoxicated birds also revealed degenerative changes with increased mucous production. The birds succumbed during the experimental period revealed degeneration and desquamation of epithelial lining of the mucosal folds with presence of cellular debris in the lumen of proventriculus. The proventriculus of intoxicated birds died at the end of study period revealed hyperplasia of the parietal cells, submucosal edema with mononuclear cell infiltration in lamina propria, necrosis and dilated crypts (Fig: 5)

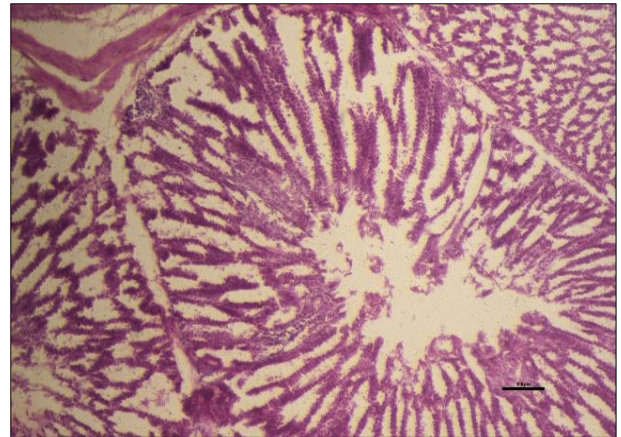


Fig 5: Photomicrograph of proventriculus revealed necrosis and dilated crypts

Gizzard: Microscopically, the birds of control group revealed well developed glandular structure covered by thick keratinized layers. The glandular epithelium of mucosa of gizzard in nimesulide intoxicated birds revealed marked depletion of keratinized layer which might be due to excessive acid production in the proventriculus as a result of hyperplasia of the parietal cells. At the end of study period, the gizzard of intoxicated birds revealed desquamation of glandular epithelium and complete degeneration of epithelium forming necked basement membrane in the gizzard (Fig: 6).

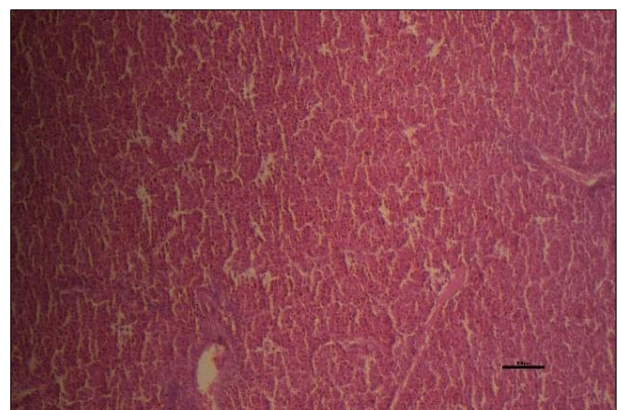


Fig 6: Photomicrograph of gizzard revealed desquamation and degeneration of epithelium

Brain: Microscopic examination of the brain tissue of intoxicated birds showed leptomenigeal congestion along with perivascular and perineuronal edema in cerebral cortex. There were neuronal degeneration and necrosis associated with gliosis especially in the perivascular areas together with satellitosis and neuronophagia (Fig: 7). The necrotic cells appeared shrunken and stained darker with haematoxylin.

After one week of intoxication, the brain of succumbed birds revealed varying degrees of congestion involving meningeal vessels, cerebral microvasculature and cerebellum. At the end of experimental period, the brain of intoxicated birds revealed perivascular oedema and neuronal degeneration characterized by shrinkage, basophilia with increased perineuronal space, gliosis and satellitosis.

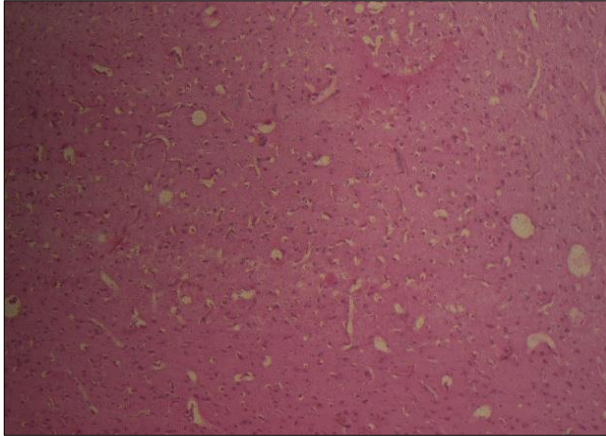


Fig 7: Photomicrograph of brain revealed satellitosis and neuronophagia

Crop: Crop revealed generalized edema, marked degeneration of mucosa and presence of lymphoid aggregates in the walls. The proliferation of stratified squamous epithelium and desquamation of mucosa were a consistent feature observed throughout the study period. After the first week of intoxication, the crop muscles of intoxicated birds revealed fatty and hyaline degeneration. The birds succumbed during the study period revealed epithelial degeneration, denudation of mucosa and oedema. At the end of experimental period the crop of intoxicated birds revealed hyperplastic changes, desquamation of the mucosa and lymphocyte infiltration in the ganglia of crop.

Thyroid: No changes were observed at day 7. Birds sacrificed at day 15 revealed enlarged thyroid follicles filled with colloid material. Frequently follicles appeared dilated with degeneration of glandular epithelium (Fig: 8).

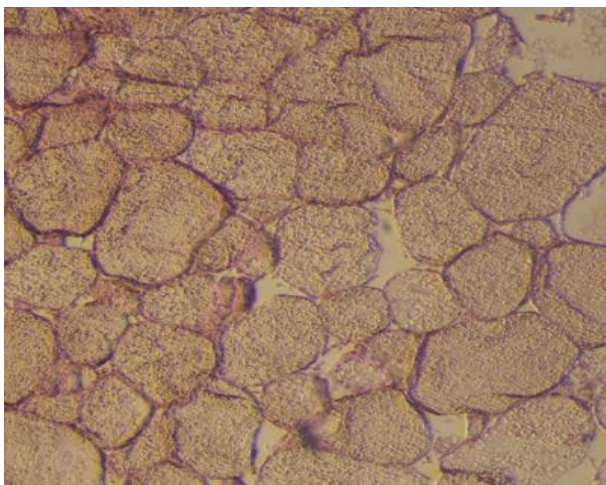


Fig 8: Photomicrograph of thyroid revealed enlarged thyroid follicles

Joints: Microscopically, the succumbed birds in the toxicity group revealed moderate deposition of white viscid material on the articular surfaces around the area of soft tissue along

with tendon sheath of the joints. Hock and toe joints were most commonly affected throughout the study period, however some deposits of urates were rarely observed in the cervical articulations.

Spleen: Microscopic examination of the spleen of the intoxicated birds revealed proliferation and desquamation of endothelial lining of the central artery of the white pulp. In some cases central arteries showed fibromuscular dysplasia characterized by massive proliferation of endothelial cells. After ten days of intoxication, the spleen revealed vascular congestion, hemorrhages, necrosis, moderate rarefaction of periarteriolar lymphoid sheath and histiocytosis. At the end of experimental period, the spleen of intoxicated birds revealed moderate depletion of lymphoid tissue in white pulp and necrotic masses in association with cellular hyperplasia (Fig: 9).

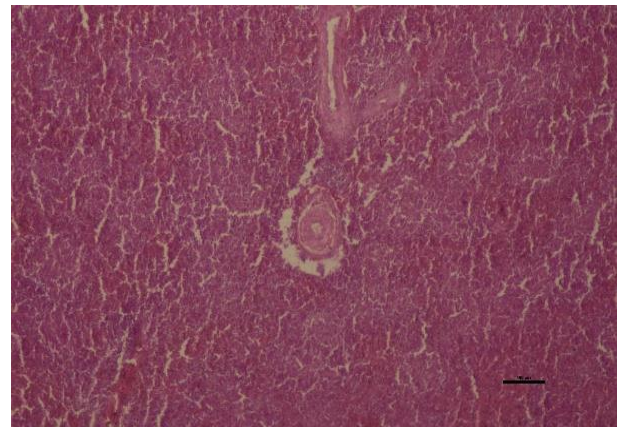


Fig 9: Photomicrograph of spleen revealed lymphoid depletion in white pulp

Bursa: No changes except mild vascular congestion was noted in the first week of experimental period. At day 10 of intoxication, the bursa of fabrics revealed moderate vascular congestion, interstitial oedema, degenerative changes along with depletion of lymphoid cells were predominant in bursal follicles (Fig:10). In few cases, necrosis and hemorrhages were also evident in the bursal follicles. At the end of study period, lysis out of bursal lymphoid cells were characterized by presence empty spaces surrounded by thick band of the connective tissue which were frequently observed in severe cases.

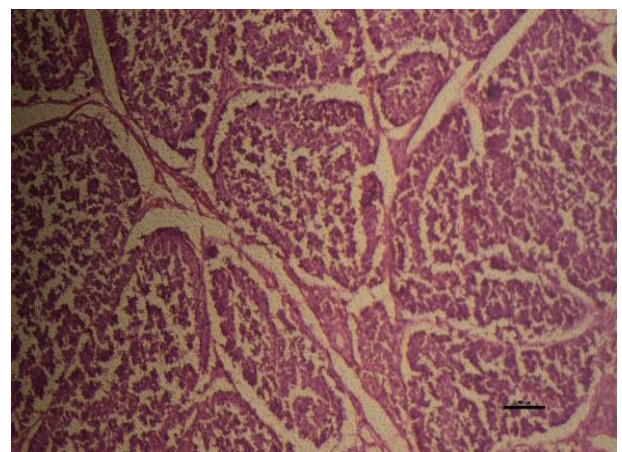


Fig 10: Photomicrograph of bursa revealed atrophy and lymphoid depletion

Thymus: Microscopic examination revealed decrease in the size of thymic follicles and depletion of lymphoid tissue from the thymus. The thymus of intoxicated birds also revealed congestion and hemorrhage throughout the study period. In some cases, there were complete degeneration of thymic lobules, hypoplasia and proliferation of interstitial fibrous tissue. At the end of experimental period, the thymus of intoxicated birds revealed atrophy and severe lymphoid depletion from the lymphoid follicles of thymus (Fig: 11).

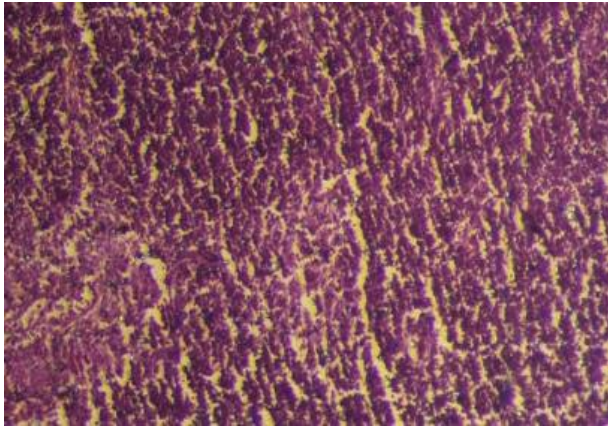


Fig 11: Photomicrograph of thymus revealed lymphoid depletion

Discussion

Nimesulide is a non-steroidal anti-inflammatory drug commonly used to reduce pain in various diseases and postoperative conditions. Their anti-inflammatory effect is mainly due to the inhibition of the activities of cyclooxygenases enzymes which interferes with the production of prostaglandins from arachidonic acid. The inhibition of prostaglandin synthesis is an important factor which contributes to the gastrointestinal toxicity in humans as well as in animals.

The clinical signs were more evident in birds after 10 days of post intoxication. The intoxicated birds revealed depression, drowsiness, in appetite, huddling together, Diarrhea, dehydration, laboured breathing and lowering of head (Mori *et al.*, 2000) [6]. Broiler chicks showed the observer able clinical signs like anorexia, emaciation, depression, feather picking, swollen and painful joints after the oral administration of non-steroidal anti-inflammatory drug (Patel, 2005) [10]. At the end of the experimental period, the intoxicated birds which survived for few days revealed retarded growth, anorexia, watery droppings, muscle weakness, staggering gait and loss of response to stimulus (Roder, 1996) [12]. The most predominant gross lesions observed in liver were yellowish discoloration which was accompanied with enlargement, petechial hemorrhages, congestion, necrosis, swollen hepatocytes and cooked appearance in many cases of intoxicated birds (Oaks *et al.*, 2004) [9]. The kidneys of the affected birds were generally rough, mottled, Necrotic and ecchyotic hemorrhages were found on the surface. On microscopic examination, the kidneys of intoxicated birds revealed marked interstitial nephritis characterized by infiltration of mononuclear cells, comprising of macrophages and lymphocyte in the interstitium (Gajera, 2006) [2]. The lungs of the intoxicated birds revealed congestion, oedema, consolidation, emphysematous and the focal areas of bronchopneumonia in association with cellular infiltration particularly lymphocytes in air vesicles, bronchi and bronchioles (Mir *et al.*, 2005) [1].

Postmortem examination of the intoxicated birds revealed enlargement of the heart, ecchymotic hemorrhages, pericarditis and necrotic foci on the organ surface throughout the study period (Arun *et al.*, 2004) [1]. Proventriculus generally appeared enlarged, edematous and congested with serous exudate present in the lumen throughout the experimental period. Microscopically, the intoxicated birds revealed degeneration, hyperplasia of the parietal cells and desquamation of epithelial lining of mucosal folds with presence of cellular debris in the glandular lumen of proventriculus (Gajera, 2006) [2]. The Gizzard of intoxicated birds revealed discolouration, hemorrhages and necrotic spots on the surface of the organ. Microscopically, the glandular epithelium of mucosa of gizzard of intoxicated birds revealed marked depletion of keratinized layer which might be due to excessive acid production in the proventriculus as a result of hyperplasia of the parietal cells (Mir *et al.*, 2005) [1]. The gross lesions of the nervous tissue comprised hemorrhagic lesions, pale discoloration, oedema and necrotic spots on the brain surface. Microscopic examination of the brain tissue of intoxicated birds showed leptomenigeal congestion, perineuronal edema, neuronal degenerations, gliosis, satellitosis and neuronophagia (Marzouk *et al.*, 2006) [5]. The intoxicated birds revealed swollen hock and toe joints resulting in the lameness of the birds. The joints of intoxicated birds further revealed the presence of viscid material on the articular surfaces of the bones (Nayak *et al.*, 1999) [8]. The spleen of intoxicated birds are enlarged, congested, mottled and appeared necrotic. Microscopically, the birds died during the experiment revealed moderate depletion of lymphoid tissue in the white pulp of spleen and desquamation of endothelial lining in the central artery (Rao *et al.*, 1993) [11]. Hemorrhages were more prominent in bursa of Fabricius of intoxicated birds which was easily visualized on gross examination. Microscopically, Bursa revealed vascular congestion, hemorrhages, interstitial oedema and depletion of lymphoid cells from the bursa (Khurana *et al.*, 2005) [3]. The Thymus of intoxicated birds appeared reduced in size, discolored, congested, edematous and necrotic. Microscopic examination revealed decrease in the size of thymic follicles and lymphoid depletion along with the enlargement of the thyroid follicles (Songur, 2005) [13].

Conclusion

From this investigation it is concluded that Nimesulide caused marked pathomorphological changes in the visceral organs and depletion of lymphoid tissue from the lymphoid organ which were suggestive of immunosuppressive effect of Nimusilide in intoxicated birds.

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