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RESEARCH ARTICLE

Pathomorphological study of Flunixin meglumine toxicity in broiler chicks

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Abstract : The aim of the present study was to evaluate the toxicopathological effects of flunixinmeglumine in broiler chicks. The chicks of group I were kept as control while group II, III and IV were fed with diet containing flunixinmeglumine @ 10 ppm, 25 ppm and 50 ppm, respectively for 21 days. Clinical signs *viz.*, dullness, depression, anorexia, shifting lameness, unthriftiness with ruffled feather, drooping of the wings and lethargy with shrunken eyes were noticed in birds of treatment group III and IV only. Maximum mortality was observed in group IV (48%) followed by group III (20%) and group II (4%). A dose dependent reduction in body weight was observed in all treatment groups. The mean values of kidney: body weight ratio was significantly increased in all treatment groups. The plasma uric acid values were significantly increased in treatment group II whereas highly significantly increased in group III and IV. The plasma creatinine and BUN values were significantly increased in treatment group III, whereas, highly significantly increased in group IV. Grossly, chalkywhiteurate deposits of varying degree on mucosal or serosal surface and parenchyma of visceral organs and joints were observed in chicks that died during experiment from treatment group III and IV. Microscopically, the lesions were characterized by congestion, haemorrhages, degeneration, necrosis and deposition of urate crystals in visceral organs. The overall lesions gave an impression that flunixinmeglumine was nephrotoxic in nature and causes similar toxicity in broiler chicks as diclofenac does in vultures.

Key words : Biochemical, Broiler chicks, Flunixinmeglumine (FM), Kidney, Nephrotoxicity, Urate crystals, Visceral gout

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INTRODUCTION

Veterinary treatment of livestock with diclofenac, a non-steroidal anti-inflammatory drug (NSAID) cause kidney failure and visceral gout and was the only cause of catastrophic declines of *Gyps* vultures in Asia, was first time indicated by Oaks *et al.*, 2004. In May, 2006 the Drug Controller General of India withdrew all licenses to manufacture diclofenac for veterinary use (Kumar, 2006). After the ban of diclofenac in veterinary field other NSAIDs such as flunixin, ketoprofen, carprofen, meloxicam etc. are now commonly used in veterinary practice. Flunixin meglumine, ketoprofen and carprofen have COX-1 and COX-2 actions (Swan *et al.*, 2006), and it is well recognized that these

NSAIDs were capable of producing potent analgesia in both mammals (Nolan, 2000) and birds (McGeown *et al.*, 1999). Flunixin meglumine is used alone or in combination with antimicrobial agents to treat a variety of diseases in domestic animals like, lameness, colic, endotoxemia, mastitis, metritis and respiratory diseases.

Diclofenac, and now ketoprofen have been clearly shown to be toxic to *Gyps* vultures, and carprofen and flunixin are also likely to be toxic (Cuthbert *et al.*, 2007). Therefore an alternate NSAID was needed to replace diclofenac in veterinary medicine which might, at least be equally efficacious but safer for the scavenging birds. This suggests that the FM may be a potential alternative of diclofenac for treatment of sick domestic animals. It is difficult to do experimental work with vultures hence, the commercial broilers were being exploited as an experimental model for screening NSAIDs used in veterinary practice to suggest better alternative to diclofenac which might prove safer to vultures. It is also necessary to investigate whether broiler chicks were susceptible to FM toxicity or not because poultry feed also contain many animal source by products like meat and bone meal, blood meal, mutton tallow etc. which are likely to have FM residue and probably responsible for visceral gout in broilers also.

RESEARCH METHODOLOGY

A total of 100 apparently healthy day old Cobb-400 broiler chicks were procured from a local commercial hatchery (Shakti Broiler Breeders Pvt. Ltd., Sarsa, Anand, Gujarat, India) and were maintained at experimental unit, Department of Veterinary Pathology, College of Veterinary science and Animal husbandry, Anand under standard managemental conditions. The chicks were given feed and fresh water *ad libitum.* Chicks were randomly divided into 4 equal groups. Broiler chicks were fed graded dose of FM (Virbac animal health India Pvt. Ltd., Mumbai, Batch number VB107) through dietary inclusion for 21 consecutive days at the dose rate of 10, 25 and 50 mg/kg (ppm) of feed in group II, III and IV, respectively, while group I was offered feed free of FM and served as control. All the birds were observed daily for any abnormal physical or behavioural changes and mortality throughout the period of 21 days of experiment. Weighing of chicks was carried out at day 1 and at the end of every week. At the end of experiment *i.e.* on 22nd day, about 2 ml blood was collected from jugular vein in vaccutainer having K_3EDTA (1-2 mg/ml) as an anticoagulant for plasma separation. Plasma samples were stored in deep freeze at -20°C for further uric acid, creatinine and BUN estimation. After the blood collection all the surviving birds from all four groups were subjected to a terminal sacrifice and detailed post mortem examination was performed. The gross pathological lesions were recorded and for histopathological examination, tissue from kidney, liver, heart, lung, spleen, proventriculus, gizzard and intestine were collected in 10 per cent formalin. Further the kidney, liver and heart tissues were fixed in absolute alcohol for De-Galantha's special staining for demonstration of urate crystals (Luna, 1968).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Clinical signs :

Chicks in the group III and IV showed similar clinical signs, which are gradually more pronounced in group IV chicks. Birds appeared emaciated, dehydrated, depressed and lethargic with shrunken eyes. They also exhibited signs like anorexia, feather plucking and uneven in growth. Birds exhibited a tendency to remain standing at one place with apathy, unthriftiness with ruffled feather, dullness and drooping of the wings. Respiration of affected birds was deep and rapid just before death. Prior to death, some of the birds showed change in temperament whereas some died suddenly without manifesting any altered behaviour. Moreover, involvement of joints was observed in some of the birds. Such birds showed shifting leg lameness and inability to stand. Affected joints were warm, swollen, tender and painful.

Mortality :

Maximum mortality was observed in group IV (48%) followed by group III (20%) and group II (4%). In group

IV maximum mortality (about 75% of total mortality) was noticed from day 8 to day 13 of the experiment whereas in group III mortality started from day 10 and continued up to day 14 of the experiment (Table 1).

Body weight gain (BWG) :

At the end of 1st week only group IV showed a significant decrease in BWG as compared to control. The group III and IV showed significant and highly significant reduction in body weight, respectively at the end of 2nd week as compared to control group. At the end of 3rd week of experiment group II showed significant whereas, group III and IV showed highly significant reduction in BWG as compared to control (Table 2).

Kidney: body weight ratio :

The mean values of kidney:body weight ratio was significantly increased in group II whereas, group III and group IV showed highly significant increase in kidney:body weight ratio as compared to control (Table 1).

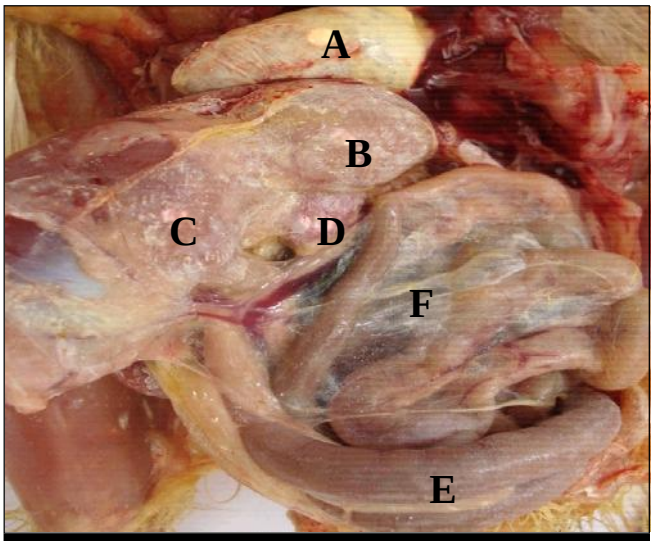

Fig. 1 : Chalky white urate deposition on surface of pericardium (A), proventriculus (B), gizzard (C), spleen (D), intestine (E), air sac (F) in group IV dead bird

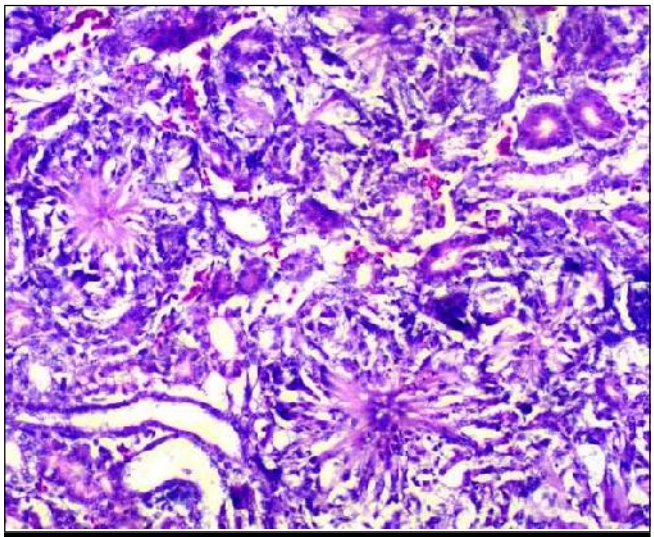

Fig. 2 : Kidney from group IV dead bird showing haemorrhage, necrosis and deposition of variable sized uric acid crystals in radiating rosette pattern with inflammatory cell infiltration. (H and E × 150)

Table 1 : Mortality and Kidney : body weight ratio (mean ± SE, $\times 10^{-3}$) of broiler chicks of different experimental groups at the end of treatment period

Experimental group	No. of birds died	Mortality %	Kidney: Body weight ratio
I	0	0	6.675 ± 0.270
II	1	4	7.312 ± 0.304*
III	5	20	8.045 ± 0.394**
IV	12	48	10.519 ± 0.509**
Total deaths	18		

* and ** indicate significance of values at P = 0.05 and P=0.01, respectively

Table 2 : Comparison of cumulative body weight gain (mean ± SE, g) of broiler chicks of different experimental groups

Days	Group – I	Group – II	Group – III	Group – IV
0	50.04 ± 0.21	49.96 ± 0.23	49.88 ± 0.25	49.69 ± 0.27
7	139.04 ± 2.24	138.36 ± 2.23	137.52 ± 2.11	122.91 ± 2.32*
14	323.53 ± 2.97	320.04 ± 3.18	314.60 ± 3.26*	289.88 ± 3.13**
21	662.47 ± 4.41	632.67 ± 5.44*	582.50 ± 4.08**	509.57 ± 3.55**

** and * indicate significance of values at P=0.01 and P=0.05, respectively

Plasma biochemical parameters :

Increased mean values of plasma uric acid, creatinine and BUN were noticed in all treatment groups as compared to control (Table 3). The chicks of group II revealed significant increase, whereas, the chicks of group III and IV revealed highly significant increase in plasma uric acid as compared to control. The chicks of group III revealed significant and chicks of group IV revealed highly significant increase in plasma creatinine and BUN as compared to control chicks.

Pathomorphology :

Grossly chalky white urate deposition were seen over entire viscera after opening the abdomen in birds died during experiment from group IV (Fig. 1).

Kidney :

Grossly, all birds of treatment groups which died during experiment revealed lesions of visceral gout. The birds of group III died during experiment revealed pale, slightly swollen kidney with dilated tubules filled with uric acid crystals and uraters were distended with semisolid to semifluid chalky white urates giving cord like appearance to ureters. Kidneys of group IV dead birds were more enlarged with pinpoint haemorrhages and tubules were studded with uric acid crystals giving them a frosted appearance with dilated ureters (Fig. 5).

Histopathologically, kidneys of bird died during experiment from group II revealed mild congestion and degeneration of renal tubular epithelium while, the kidneys of dead birds of group III and IV showed congestion, parenchymatous degeneration and necrosis of renal tubular epithelium. Moreover, kidney of group IV birds also revealed cystic dilatation of renal tubules, complete destruction of glomeruli and desquamation of tubular epithelium with presence of proteinacious cast in renal tubular lumen. Abundant deposition of colourless to basophilic, variable sized uric acid crystals with inflammatory cell infiltration were present in renal parenchyma, assuming radiating crystalline rosette pattern (Fig. 2). The urate deposits gave positive reaction with De-Galantha's stain and appeared as black needle shaped crystals, assuming radiating rosette pattern in chicks died from group IV (Fig. 6). Kidney from the sacrificed birds of group I, II and III did not reveal any abnormal lesions. Tubular epithelial degeneration was noticed in high dose group chicks only. However, the severity of the lesion was less in sacrificed chicks as compared to chicks died during experiment in the same group.

Liver :

Grossly, livers of group III and IV dead chicks were enlarged, soft, friable and congested as compare to control.

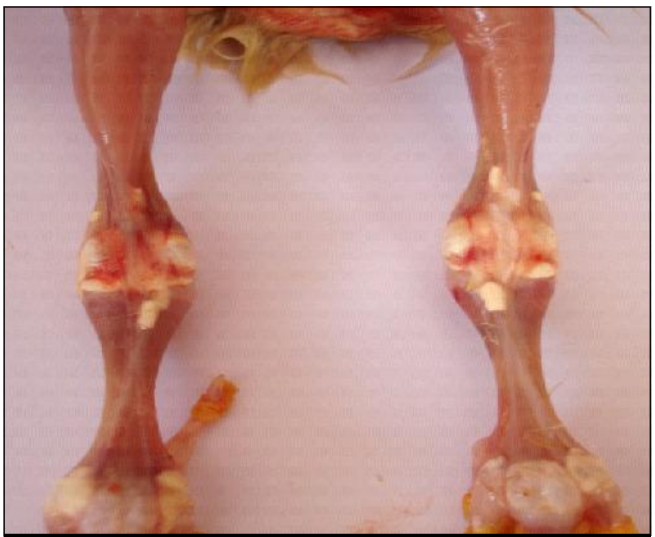

Fig. 3 : Uric acid deposition in hock and phalangeal joints of group IV dead bird

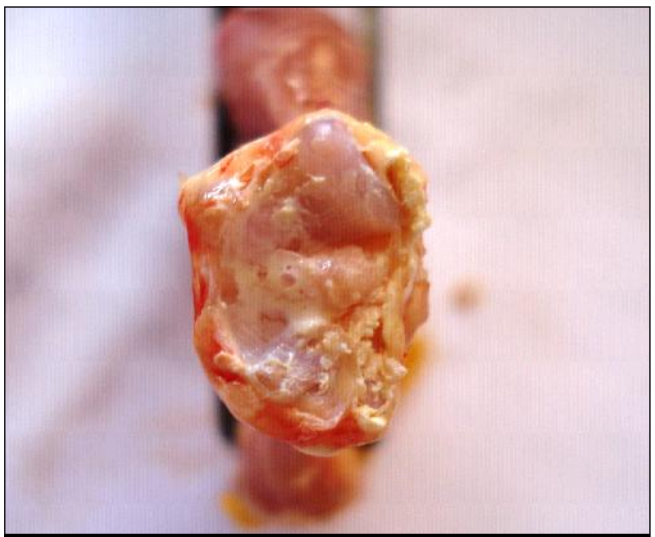

Fig. 4 : Uric acid deposition on articular surface of hock joint of group IV dead bird

In most of the cases, varying degree of chalky white urate deposits covered the serosal surface of liver in chicks died during experiments from treatment group III and IV.

Histopathological changes in liver of chicks died from group III revealed congestion, degeneration and necrosis with multifocal urate deposition in hepatic parenchyma. Lesions were more severe in liver of group IV birds with severe congestion, haemorrhage, degeneration and necrosis with deposition of urate tophi surrounded by infiltration of inflammatory cells, the predominant cell type being heterophils and mononuclear cells (Fig. 7). Hepatic parenchyma showed deposition of black coloured radiating rosette uric acid crystals with De-Galantha's staining in died chicks from group IV (Fig. 8).

Heart :

Grossly mild to severe deposition of chalky white urate on the pericardium of heart, which was firmly adhered to heart (Fig. 1) in chicks died from group III and IV.

Microscopically, myocardium of the heart showed congestion, haemorrhages and urate deposition surrounded by inflammatory cell infiltration along with destruction of myocardial cells in chicks died during experiment from group III and IV. Chicks died during experiment from group III and IV showed black coloured urate deposition either in

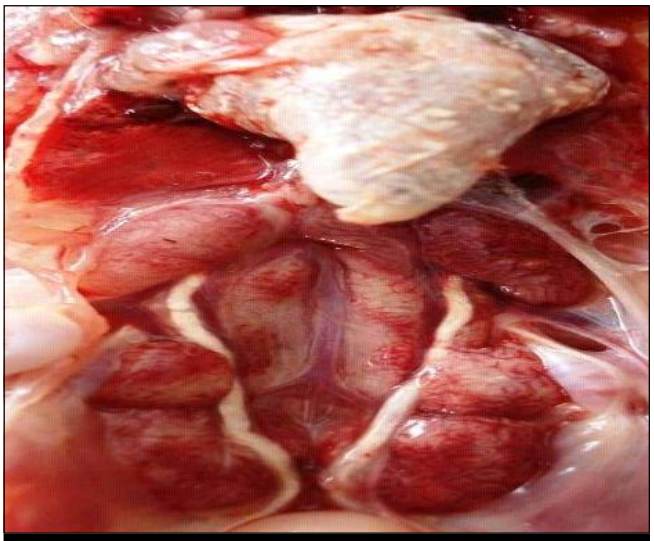

Fig. 5 : Group IV dead bird showing pale swollen and frosted kidney with dilated ureter studded with uric acid

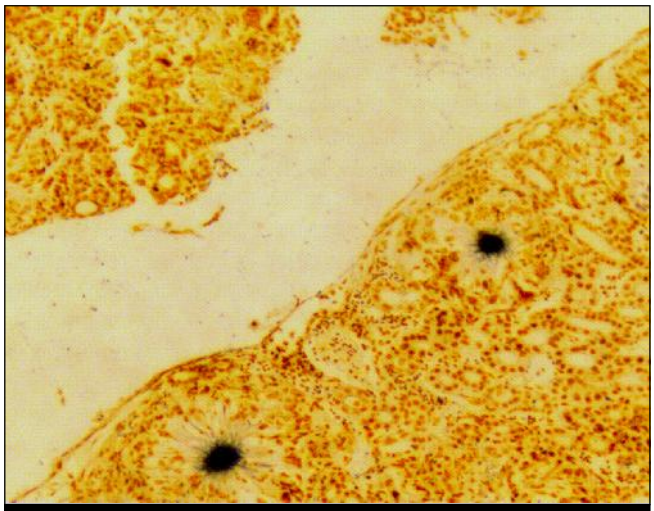

Fig. 6 : Kidney from group IV dead bird showing black coloured radiating rosette uric acid crystals. De-Galantha's stain × 150

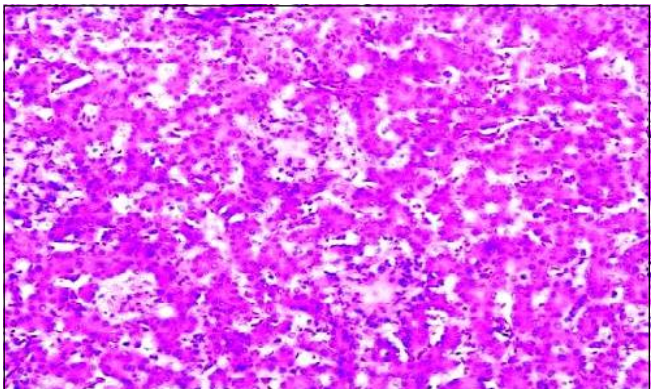

Fig. 7 : Liver from group IV dead bird showing degeneration with uric acid deposition surrounded by inflammatory cell infiltration. H & E × 150

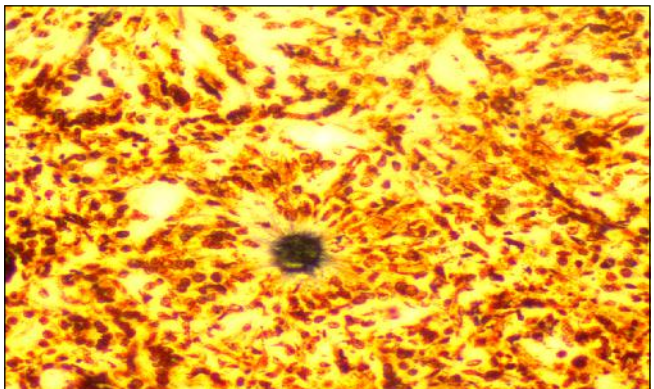

Fig. 8 : Liver from group IV dead bird showing black coloured radiating rosette uric acid crystals deposition. De-Galantha's stain × 150

amorphous material and/or radiating rosette pattern in epicardium and myocardium of the heart section stained with De-Galantha's stain (Fig. 9).

Lung :

Grossly, lungs were congested in most of the cases in treatment group III and IV. Most of the birds died during experiment from treatment group IV showed deposition of urate crystals on pleural and serosal surfaces of lungs.

Histopathologically, lung from group III and IV dead birds revealed congestion, mild to severe haemorrhages and focal area of necrosis with multifocal urate deposition which was surrounded by inflammatory cell infiltration in lung parenchyma (Fig. 10). The chicks sacrificed at the end of experiment from group IV revealed only mild congestion.

Intestine :

Grossly, there was deposition of white urate crystals over serosal surface of intestine (Fig. 1) in chicks died during experiment from group III and IV. Moreover, whole length of intestine was firmly adhered to itself because of urate deposition on the mesentery and visceral peritoneum in chicks died during experiment from treatment group III and IV.

Microscopically, intestinal section from group IV showed mild degeneration and necrosis of tip of villi with congestion and haemorrhages.

Joints :

Grossly, no appreciable changes were observed in joints of birds sacrificed at the end of experiment. Most of chicks died during experiment from treatment group IV showed white uratedeposition in hock and phalangeal joints

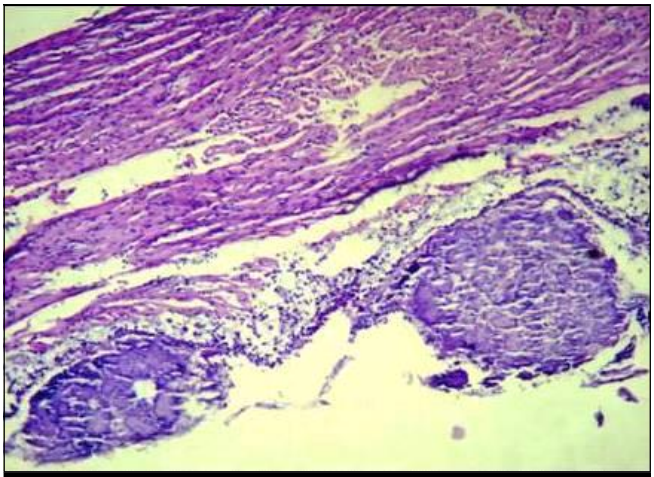

Fig. 9 : Heart from group IV dead bird showing amorphous uric acid deposition on pericardium. H & E × 60

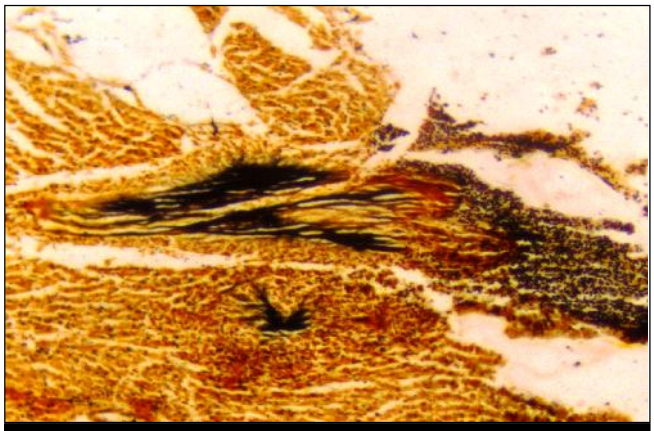

Fig. 10 : Heart from group IV dead bird showing black coloured uric acid deposition in epicardium and myocardium in amorphous and radiating crystalline pattern. De-Galantha's stain × 150

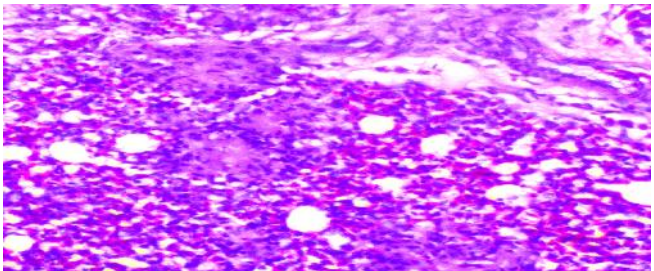

Fig. 11 : Lung from group IV dead bird showing haemorrhage and uric acid deposition with inflammatory cell infiltration. H & E × 300

Table 3 : Mean values (± SE) of plasma uric acid, creatinine and BUN of broiler chicks of different experimental groups

Experimental group	Uric acid (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)
I	6.027 ± 0.21	0.374 ± 0.02	1.695 ± 0.04
II	6.756 ± 0.24*	0.407 ± 0.05	1.75 ± 0.07
III	12.118 ± 0.32**	0.50 ± 0.04*	2.121 ± 0.09*
IV	23.332 ± 0.44**	0.885 ± 0.04**	2.735 ± 0.08**

** and * indicate significance of values at P=0.01 and P=0.05, respectively

(Fig. 3). On opening of the hock joint, articular surface showed uric acid deposition (Fig. 4). Similar lesions were also present in few birds died during experiment from group III but with less severity as compared to group IV. Few birds died during experiment from group IV showed urate deposition in wing joints also.

The present study indicated that the compound FM could produce clinical signs like dullness, dehydration, anorexia, uneven growth, feather plucking, lethargy and shifting leg lameness were in agreement with the findings of earlier workers (Pereira and Werther, 2007; Muhammad *et al*., 2012). Similar clinical signs were also reported by earlier workers (Seema, 2006; Shinde, 2008 and Irtaza *et al.*, 2008) during their study on diclofenac toxicity in broiler chicks. The mortality observed in present study was in agreement with report of earlier workers (Muhammad *et al*., 2012; Cuthbert *et al.*, 2007). Similar pattern of mortality was also reported by earlier worker during their study on ketoprofen and aceclofenac intoxication in broiler chicks (Undhad *et al.*, 2013 and Patil, 2013). The present study also suggested that older birds were less sensitive to FM or that surviving birds were able to resist FM toxicity more efficiently.

The observations of decrease in BWG were in close association with the previous studies (Seema, 2006; Irtaza *et al.*, 2008 and Shinde, 2008) on diclofenac toxicity in broilers. The results of decrease in BWG were also reported by earlier worker during their study on ketoprofen and aceclofenac toxicity in broiler chicks (Patil, 2013 and Undhad *et al.*, 2013).The results of increase in kidney:body weight ratio were in alliance with earlier worker (Seema, 2006; Undhad *et al.*, 2013 and Patil, 2013) in their study on diclofenac, ketoprofen and aceclofenac toxicity in broilers. The present study suggested that mean kidney:body weight ratio was higher due to reduced body weight and increased kidney weight because of urate deposition and inflammatory reactions in higher dose groups.

The biochemical investigation revealed that the increase in level of uric acid, creatinine and BUN were in consonance with findings of earlier worker (Muhammad *et al*., 2012). Intoxication of NSAIDs like diclofenac, ketoprofen and aceclofenac also cause increase level of uric acid, creatinine and BUN in broiler or layer chicks as reported by earlier workers (Seema, 2006; Irtaza *et al.*, 2008; Shinde, 2008; Jain *et al.*, 2009; Ghanvat, 2012; Patil, 2013; Undhad *et al.*, 2013 and Patel *et al.*, 2014). Increased uric acid, creatinine and BUN might be due to compromised kidney functions and renal failure caused by FM toxicity, which leads to failure of excretion of uric acid and resulted in hyperuricaemia. So that blood became supersaturated with uric acid and it was precipitated in the kidney, mucosal or serosal surfaces, parenchyma of visceral organs and joints in the form of urate crystals. Cellular reaction to these crystals further provoked inflammatory changes in the kidneys and ultimately resulted in visceral gout.

Grossly, white chalky urate deposits of varying degree on surface of visceral organs and joints were observed in the present study is in agreement with the previous observations made by earlier worker (Muhammad *et al*., 2012). Mir *et al.*, 2005; Seema, 2006; Irtaza *et al.*, 2008; Shinde, 2008; Jana *et al.*, 2009; Sharma and Vegad, 2010 and Undhad *et al.*, 2013 reported urate deposition on serosal or mucosal surface and parenchyma of visceral organs due to diclofenac, ketoprofen toxicity in broiler or layer chicks. Severity and distribution of the pathological lesions was dose dependent. Histopathologically, congestion, haemorrhage, degeneration, necrosis and deposition of urate crystals in kidneys, liver, heart, spleen, lung and intestine were also reported by various workers (Seema, 2006; Irtaza *et al*., 2008; Shinde, 2008; Jain *et al.*, 2009; Jana *et al*., 2009; Sharma and Vegad, 2010 Muhammad *et al*., 2012; Undhad *et al.*, 2013 and Patil, 2013). Klein *et al.*,1994 and Hocking *et al.*, 2005 reported renal lesions in Bobwhite quail treated with FM.Clyde and Paul-Murphy, 1999 also reported renal ischemia and necrosis in Siberian cranes treated with FM at the dose rate of 5 mg/kg. This suggests the main target organ affected due to flunixin toxicity was kidneys.

The overall lesions gave an impression that FM was nephrotoxic in nature and is primarily cleared by the kidneys. Histopathological findings of the present study in kidneys are corresponding with the biochemical finding like increased levels of plasma uric acid, creatinine and BUN which causes decrease in glomerular filtration rate of FM and its metabolite from kidney, leading to kidney lesions and ultimately visceral gout.

The plasma biochemical alterations and the pathological changes observed during present investigation on toxicopathological studies of FM in broiler chicks clearly indicated that FM is nephrotoxic to broiler chicks. The pathological changes observed were similar to that of diclofenac toxicity in broiler chicks. It was presumed that treating livestock with FM might have similar residual toxicity of diclofenac to the vultures. Patil *et al.*, 2012 and Mane *et al.*, 2012 also worked on the related topic.

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