V. DISCUSSION

5.1 Spectroscopic and chromatographic characterization of NSAIDs

5.1.1 UV visible spectroscopy

In the present study, the absorption wavelength and the peak shape obtained from the recorded UV visible spectrum was characteristic for the drugs; diclofenac sodium, aspirin, paracetamol, ketoprofen, nimesulide, meloxicam and celecoxib. This was further, reasserted by matching the absorption wavelength of individual NSAID with the absorption wavelength of the reference spectra (Moffat, 2006).

5.1.2 Infrared spectroscopy

Infrared spectroscopy has been extensively used in both qualitative and quantitative pharmaceutical analysis (Aldrich and Smith, 1999; Bugay, 2001; Clark, 2002).

This technique considered to be important for the evaluation of the raw materials used in production, the active ingredients and the excipients (the inert ingredients in a drug formulation, e.g. lactose powder, hydroxypropyl cellulose capsules, etc.). Infrared spectroscopy provides valuable additional structural information, such as the presence of certain functional groups. The presence of functional groups in the structure is characteristic for particular compound (Stuart, 2004).

The infrared spectrum mainly broken down into three major regions; 1. The functional group region (1600-4000 cm\(^{-1}\)) in which most functional groups absorb. 2. The fingerprint region (1000-600 cm\(^{-1}\)) often used for band-by-band comparison of the spectrum of a known compound to the spectrum of an unknown compound in order to identify the compound. 3. The aromatic region (675-900 cm\(^{-1}\)) useful for identifying the number and relative positions of groups on a benzene ring (Stuart, 2004).
In the present study, the principal wave numbers obtained from the recorded infrared spectrum was characteristic for the drugs; diclofenac sodium, aspirin, paracetamol, ketoprofen, nimesulide, meloxicam and celecoxib. This was further, reasserted by matching the principal wave numbers of infrared spectrum for individual NSAID with the wave numbers of the reference spectra (Moffat, 2006)

Further, the infrared spectrum used as a fingerprint for drug molecules. The infrared spectra of two substances when compared and matched peak for peak, it is possible to say that they are the same or identical compound (Silverstein and Webster, 2001).

Similarly, in the present study, the infrared spectrum for drugs such as diclofenac sodium, aspirin, paracetamol and ketoprofen was matched and compared with the in built library to verify the compound identity.

The infrared spectrum of all the NSAIDs was reasserted to possess similar principal wave numbers in comparison to reference spectra and thereby confirms the genuineness of the procured NSAIDs.

**5.1.3 Thin layer chromatography**

One of the most effective screening methods being used even today is the thin-layer chromatography (TLC), simplest of all the widely used chromatographic methods to perform. In the determination of this method, different chromatography systems were tested and analyzed according to the classification proposed by Moffat (1986). Accordingly, drugs were divided in three categories: acid, basic and neutrals based on their polarity and acid characteristics. This procedure considered to be important for
selection of a suitable mobile phase that is capable of resolving peaks in the respective tracks spotted with NSAID.

The chromatographic systems consisted of mixtures of the following solvents: chloroform, ethyl acetate, acetone, ammonia, formic acid, methanol and water in different ratios. Nevertheless, all the mobile phases (either individual or in combination) tested was not adequate for the proper identification of all the drugs. Therefore, with the objective to develop a single mobile phase capable of resolving all the six NSAIDs, a modification in the above systems tested was proposed. Finally, the mobile phase selected was isopropyl alcohol: n-hexane (4.9: 5.1 v/v). This system was chosen due to its sensitivity and simplicity in separating all the six NSAIDs.

The peaks obtained for NSAIDs on TLC chromatogram were characteristic with unique Rf value, such that Rf value for each NSAID differed. Therefore, with a view to obtain better resolution of peaks, it was planned to spot mixture containing six NSAIDs as a single spot on TLC plate and the plates were developed using mobile phase isopropyl alcohol - n-hexane (4.9: 5.1 v/v).

Since, the mentioned chromatographic system (isopropyl alcohol: n-hexane-4.9: 5.1 v/v) was found to be undesirable for aspirin an alternative system was developed using methanol: toluene (1: 1 v/v).

All NSAIDs showed good resolution and sharp peaks beside, the spots on the developed TLC plates were found compact and not diffused which are characteristic indications of good chromatographic system.

The mobile phase consisting of isopropyl alcohol: n-hexane (4.9: 5.1 v/v) worked out to be good in separating mixture containing several NSAIDs. This was evident from
the TLC visulizer where in each compact spot on the TLC plate represents an NSAID. All the NSAIDs showed good resolution with sharp peaks. Therefore, the mentioned mobile phase can be used for identification of NSAIDs in drug formulations that are available in fixed dose combination such as diclofenac and paracetamol or nimesulide and paracetamol. Moreover, the system developed has found to be advantageous and more economic compared to conventional system where in, different mobile phases are used for separation or identification of NSAIDs (Moffat, 2006). Beside, this method can also be exploited for identification of multiple NSAIDs in serum samples of veterinary patients administered with two or more NSAIDs.

5.2 Clinical signs of toxicity

In the present study, the observed clinical signs of toxicity observed for the diclofenac received birds corroborate with findings of Swetha et al. (2005). Where in similar forms of clinical signs of toxicity were observed in broiler chickens 24 h post administration of diclofenac (2.5 mg/kg, IM). Similar signs of toxicity were also observed in the Leghorn layers wherein, the affected birds appeared to be comatose and could not be aroused. Additionally, malformation and very thin shelled eggs were noticed 24 h post administration of diclofenac (Naidoo et al., 2007).

Swan et al. (2006a) noticed lethargy and different degrees of neck drooping in vultures at 24 h post oral administration of diclofenac (2.5mg/kg). Furthermore, they also observed similar clinical signs of toxicity in vultures upon feeding tissues from goats (Capra aegagrus hircus) and buffaloes (Bubalus bubalis) treated with diclofenac few hours before slaughter. The clinical signs of toxicity observed in the diclofenac treated group are in conformity of earlier findings.
5.3 Mortality

Four out of 6 birds that died in the diclofenac group during the experimental period is suggestive of potential toxic effect evoked by diclofenac inducing high mortality (66.66%) in the exposed birds. Similarly, high mortality was also reported in broiler chickens (Swetha et al., 2005), Vanaraja and PB1 poultry breeds (Reddy et al., 2006) and Leghorn layers (Naidoo et al., 2007) upon intramuscular administration of diclofenac and also noticed in vultures on post oral dosing of diclofenac (Swan et al., 2006a). Diclofenac sodium, 0.8 mg/kg was highly toxic to G. fulvus and G. africanus species of vultures and the oral LD$_{50}$ of diclofenac in G. bengalensis was found to be 0.1-0.2 mg/kg (Swan et al., 2006a).

In the present study, difference in terms of dose of diclofenac administered and mortality rate was observed in broiler chickens when compared to vultures. In case of broiler chickens, diclofenac 2.5 mg/kg was administered orally for the period of 5 days and mortality was observed intermittently, whereas, in vultures a single dose of diclofenac (0.8 mg/kg) was enough to cause mortality in all the exposed vultures within 42 h post oral dosing (Swan et al., 2006a). The variation in the mortality rate observed in broiler chickens compared to vultures might be attributed to less susceptibility of chicken to diclofenac.

Further, high mortality was also reported in the broiler chickens administered with diclofenac (2.5 mg/kg, IM) (Mohan et al., 2008a). But, no mortality was evinced in the broiler chickens administered with nimesulide (2 mg/kg, IM) or meloxicam (0.5 mg/kg, IM) (Swetha et al., 2005). Similarly, in a study by Reddy et al. (2006), wherein toxicity
of nimesulide was compared with diclofenac sodium in poultry (Vanaraja and PB1 birds) no mortality was found for nimesulide treated (2 or 5 mg/kg) group.

In the present study, mortality was absent in the birds administered with other NSAIDs like aspirin, paracetamol, nimesulide, ketoprofen, meloxicam and celecoxib. Thus, indicating these NSAIDs are safer compared to diclofenac.

From the present study, it could be construed that the cause of high mortality observed in the diclofenac treated group might be due to acute renal failure and resultant visceral gout which was further evident from the postmortem lesions and histopathology.

5.4 Hematology parameters

The significant reductions in the RBC, haemoglobin and PCV content of the blood samples on days 3, 4, 5 and 6 in group II birds administered with diclofenac, on days 4, 5 and 6 in group III birds administered with aspirin and on days 5 and 6 in group V birds are attributed to the adverse effects exerted by these NSAIDs on hemopoietic system. The major function of the red blood cells is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues (Waugh and Grant, 2001). Anaemia is defined as a reduction in the haemoglobin concentration of the blood (Moss, 1999). Reduction in the haemoglobin may be accompanied by a fall in the red cell count (RBC) and packed cell volume (Waugh and Grant, 2001). Very low readings for RBC, heamoglobin and hematocrit observed for these groups are indication of anemia possibly due to heavy loss of RBCs on account hemorrhages in the gastro intestinal tract of these birds. In humans, the deaths of over 1000 people over 20 years were attributed to phenylbutazone induced aplastic anemia (Brooks et al., 1986). Anemia was also reported in horses upon administering phenylbutazone paste (Lees and Higgins, 1987). These
reports conclusively suggest that some NSAIDs do have the potential to induce anemia which was further evident from hematological examination of blood samples of birds administered with diclofenac, aspirin and nimesulide.

Avian thrombocytes play a primary role in hemostasis in a manner similar to mammalian platelets. They may also have a phagocytic function and participate in removing foreign material from the blood (Dieterlen-Lievre, 1988; Campbell, 1988). A normal thrombocyte count ranging between 20,000 and 30,000/μl of blood is used as a general reference for most birds (Jain, 1993).

Thrombocytopenias are usually indicative of excessive peripheral demand for thrombocytes, although a depression in thrombopoiesis should be considered (Campbell, 1997). Thrombocytopenias are often seen in conditions where a combination of excessive peripheral demand for thrombocytes and in case of decreased thrombocyte production. A thrombocytosis may reflect a rebound response following hemorrhage or recovery from other conditions associated with excessive utilization of thrombocytes (Campbell, 1997).

The significant reductions in the platelet count in blood samples which was observed in latter half of the study in all the treatments groups can be attributed to impair platelet activity due to impaired thromboxane synthesis. Particularly, aspirin irreversibly acetylates the platelet cyclo-oxygenase. Since, platelets cannot regenerate cyclo-oxygenases, platelet aggregation defects caused by aspirin can last up to one week (Eyre and Burka, 1979).

Besides, there are reports relating to NSAIDs induced inhibition of TXB\textsubscript{2} generation in the blood of the dogs by more than 90% following intravenous administration of piroxicam at a dose of 0.3 mg/kg (Galbraith and McKellar, 1991).
Additionally, meloxicam was observed to be a potent inhibitor of TXB$_2$, 6-ketoPGF$_2$ and bicyclic PGE$_2$ in inflammatory exudate in an equine model of acute inflammation (Lees et al., 1991).

Phenylbutazone has been shown to inhibit PGE$_2$ and PGI$_2$ concentrations in the inflammatory exudates and TXB$_2$ generation in the blood platelets of the horses (Lees et al., 1987).

5.5 Alterations in serum biochemistry

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the enzymes present in many tissues and are useful in evaluating muscle and liver damage. The significant increase in serum AST and ALT concentration in group II birds administered with diclofenac and in group V birds administered with nimesulide on day 6 is an indication of possible occurrence of hepatocellular damage due to toxic effects exerted by diclofenac sodium and nimesulide.

Most of the creatinine originates from the nonenzymatic conversion of creatine in muscle. The creatinine thus produced is filtered by glomerular filtration. Creatinine after being filtered by glomerulus is excreted in urine. Since, it is not excreted or absorbed by renal tubules to any degree, it can be used as a rough index of glomerular filtration rate (Benjamin, 1985). Increased levels of plasma creatinine observed in the diclofenac and nimesulide groups might be related to blockade of renal vasodilatation due to nonselective inhibition of the cyclo-oxygenase by diclofenac sodium and nimesulide.

Altered levels of creatinine along with AST and ALT are considered as good indicators of muscle and liver damage. Creatinine, AST and ALT levels were significantly high in diclofenac and nimesulide groups compared to the other treatment
and control groups indicating the probable muscular and hepatocellular damage caused by diclofenac and nimesulide. It is observed that, the levels of AST, ALT and creatinine shoots up in case of hepatocellular and muscular damage (Lumeij, 1997; Lumeij and Waterhof, 1987), both values forms a good tool for assessing liver and muscle damage (Dabbert and Powell, 1993).

Alkaline phosphatase (ALP) is present in liver, bound to the cells. This enzyme is also present to some extent, in all tissues but only few organs contribute to the circulating enzyme level. This enzyme plays a role in detoxification of endotoxins in the liver mainly. The altered levels of this enzyme are observed in active liver damage, extra-hepatic and intra-hepatic biliary obstructions. Elevated levels of ALP in diclofenac, paracetamol, nimesulide and ketoprofen groups are indicative of liver damage (Lumeij and Waterhof, 1987). However, alkaline phosphatase level remained at normal levels as reported in some of avian species (Kendel and Harr, 2002; Simaraks et al., 2004) and in treatment groups that have received aspirin, meloxicam and celecoxib.

Significant increase in values of BUN (blood urea nitrogen) and uric acid from day 2 onwards observed in diclofenac treated birds correlates with findings of Swetha et al. (2005) and Reddy et al. (2006). Increase in serum uric acid concentration also has been reported in the birds died due to visceral gout (Uma, 1997). Clinical cases of gout in chickens was reported when serum uric acid level exceeded 25.5 mg/dl as compared to 4.2-6.4 mg/dl in the control birds (Uma et al., 1999) the increased level of uric acid was found as high as 140 and 160 mg/dl, at 12 and 24 h after administration of diclofenac, 0.8 mg/kg orally in G. africanus vultures (Swan et al., 2006a)
The increase in the levels of uric acid BUN and creatinine observed in the present study, following diclofenac administration might be due to renal failure which leads to hyperuricemia and gout because kidneys plays an important role in elimination of uric acid and creatinine in birds. Further, an increased serum uric acid concentration can be attributed to impaired uric acid excretion due to tubular degeneration caused by diclofenac causing renal failure in birds, leading to accumulation of uric acid in blood (hyperuricemia) and tissues forming visceral gout which might be responsible for high mortality.

Liver except immunoglobulins produces almost all proteins in the serum. The total serum protein estimation serves as an important tool for assessing the functioning of liver, malabsorption from intestine due to inflammations, malnutrition, glomerulonephropathy and acute inflammations. Besides, non-steroidal anti-inflammatory drugs are well recognized for having potentially toxic effects on the gastrointestinal tract which may lead to diarrhea. The prostaglandins PGE$_2$ and PGI$_2$ are critical for the maintenance of normal mucosal blood flow within the gastrointestinal tract (Boothe, 2001).

The significant decrease in albumin concentration in group II and group III birds and plasma protein concentrations in group II through group VII birds are suggestive of gastrointestinal and hepato-toxicity (Lumeij, 1999; Denman et al., 1983). To corroborate the above findings, severe gastro-toxicity such as ulcerations, congestion and hemorrhages observed in different parts of gastrointestinal tracts of birds administered with NSAIDs; diclofenac, aspirin, paracetamol and nimesulide could be due to inactivation of the COX (cyclo-oxygenase) enzymes. The inactivation of COX leads to a
increase in prostaglandin production which, in turn, impairs mucosal blood flow and leads to mucosal inflammation and injury leading to protein loosing enteropathy (Boothe, 2001).

Diclofenac causes a rare but potentially fatal hepatotoxicity that may be associated with the formation of reactive metabolites (benzoquinones imines) via a hepatic cytochrome P₄₅₀ catalyzed oxidation which contribute to diclofenac mediated hepatic injury (Kappus, 1986; Tang, 1999). Aspirin metabolized primarily via the hepatic enzyme glucuronyl transferase (Plumb, 2002). Also, the other NSAID, paracetamol in humans gets biotransformed to toxic NAPQI (N-acetyl-p-benzoquinone imine) metabolite found to cause fatal hepatic degeneration and necrosis (Burke et al., 2006). Thus, heavy loss of serum protein observed in the diclofenac group and in other treatment groups might be attributed to the toxic effect exerted by these NSAIDs on gastrointestinal tract and liver.

The products of arachidonic acid metabolism has diverse roles in the kidney, including the regulation of renal plasma flow (prostaglandins PGI₂ and PGE₂-vasodilate; thromboxane A₂-vasoconstricts), salt and water handling and renin release by the juxtaglomerular apparatus (Kim et al., 1999).

Moreover, blood flow to the avian kidney is different from that of mammals. In mammalian kidney, PGE₂ and PGI₂ function as renal vasodilators and regulate renal blood flow supplied primarily through the afferent arteriole (Verlander, 1997). The renal dysfunction associated with use of NSAID is principally caused by inhibition of cyclooxygenase enzymes, resulting in decreased synthesis of eicosanoids (PGE₂ and PGI₂), which are important autacoids in the regulation of renal function (Delmas, 1995).
Sodium and potassium are major and most important electrolytes present in body fluids being the major cations of extracellular fluid and that of the intracellular fluid, respectively (Pandiyan et al., 2005). About 89 per cent of the total body content of potassium is within cells. Potassium maintains acid-base balance, regulation of osmotic pressure and helps in the development of cellular membrane potentials. It also influences the contractility of smooth, skeletal and cardiac muscles (Houpt, 1996).

The proper level of potassium is essential for normal cell function. An abnormal increase of potassium (hyperkalemia) or decrease of potassium (hypokalemia) can profoundly affect the nervous system and heart and when extreme, can be fatal. The normal blood potassium level is 3.5 to 5.0 meq/L. Sodium (Na) is the major extracellular cation and it plays a role in body fluid distribution. The plasma concentration of sodium ions (extracellular) is 130 to 145 meq/L (Goldstein and Skadhauge, 2000). Higher and lower concentrations are referred to as hypernatremia and hyponatremia, respectively.

In the present study, the observed increase in the serum sodium and potassium concentration in the birds that have received diclofenac, aspirin, paracetamol, nimesulide and ketoprofen could be attributed to inhibition of renin secretion by NSAIDs, creating a hyporeninemic, hypoaldosteronemic state coupled with inhibition of prostacyclin that decreases excretion of sodium and potassium (Sandhu and Rampal, 2006). Similarly, an increase in potassium concentrations were also reported in leghorn birds and in vultures died due to diclofenac toxicity (Naidoo et al., 2007).

Renal prostaglandin production is mediated primarily by cyclo-oxygenase and plays a major role in compensatory renal haemodynamics. It has previously been described that, in overdose, NSAID, such as ibuprofen, increase fractional excretion of
potassium and cause sodium retention (Goddard et al., 2003). Thus, in the present study, the observed increase in the sodium concentrations mediated by NSAIDs; diclofenac, aspirin, paracetamol, nimesulide and ketoprofen is due to toxic effect of these drugs exerted on kidneys that might have caused renal vasoconstriction and consequent activation of the renin-angiotensin aldosterone system.

In the past, it has been suggested that a terminal increase in potassium was the cause of death in gouty fowls (Lumeij, 1994). Hyperkalemia is one of the many renal complications associated with nonsteroidal anti-inflammatory drugs (NSAIDs) (Schlondorff, 1993). Potassium homeostasis is impaired by NSAIDs through the inhibition of renal prostaglandin synthesis (Garella and Matarese, 1984), especially PGE$_2$ and PGI$_2$. These prostaglandins stimulate renal synthesis of renin and thereby influence the subsequent synthesis of aldosterone (Garella and Matarese, 1984). Induction of relative hyporeninemic hypoaldosteronism is probably the major mechanism by which NSAIDs reduce renal potassium excretion and cause hyperkalemia (Tan et al., 1979). In vitro modulation of high-conductance potassium channels in distal tubular principal cells by PGE$_2$ and PGI$_2$ has also been described (Schlondorff, 1993; Garella and Matarese, 1984). These prostaglandins increase the number of open high-conductance potassium channels and facilitate potassium secretion. Thus, NSAIDs may interrupt renal potassium secretion by reducing the open state of these potassium channels (Schlondorff, 1993; Garella and Matarese, 1984). The adverse effects of NSAIDs may be accentuated if prerenal azotemia impairs delivery of salt and water to the principal cell in the distal nephron, further reducing potassium excretion (Schlondorff, 1993; Garella and Matarese, 1984). Therefore, the present study supports this supposition that the cause of death in
diclofenac group is potassium related. Since, in birds hyperkalaemia is a known cause of bradycardia (Zandvliet, 2005).

5.6 Change in organ to body weight ratio

The significant increase in organ to body weight ratio of liver in diclofenac, aspirin, ketoprofen and celecoxib groups might be attributed to potent hepatic enzyme induction coupled with treatment related changes such as hepatocellular hypertrophy (e.g., enzyme induction or peroxisome proliferation) (Greaves, 2000; Amacher et al., 2006; Juberg et al., 2006). The increase in liver weight in the studies of less than 7 days duration are attributed to potent hepatic enzyme inducing property of test compounds (Greaves, 2000). Histological examinations of liver tissue of diclofenac received bird’s revealed necrosis and hepatocellular hypertrophy which might be attributed to toxic effect exerted by diclofenac. However, the hepatocellular hypertrophy was not observed in any of the other treatment groups. Hence, it could be construed that the observed increase in the liver weight in the other treatment groups could be due to hepatic enzyme induction by the NSAIDs that were orally administered consecutively for a period of 5 days.

Significant increase in weight of the heart observed in the diclofenac group could be due to myocardial hypertrophy (Thiedemann, 1991; Greaves, 2000). Grossly pericardium of the heart was thickened considerably and edematous due to accumulation of fluid in the pericardium. The accumulation of the fluid in the pericardium might be due to the sodium retention which was evident from the estimation of serum sodium concentration in the diclofenac group. These lesions coupled with alteration in sodium concentration could have contributed for increasing the organ weight of the heart.
Significant increase in weight of the kidneys observed in the diclofenac group could be due to renal toxicity; tubular hypertrophy or progressive nephropathy (Greaves, 2000). In confirmation of the above findings, the change in the morphological structure of kidneys contributing to increased weight was evident from gross as well as histological examinations of kidney sections of diclofenac group.

5.7 Pathological lesions

The most predictable and serious adverse effects associated with NSAIDs occur in the gastrointestinal tract (Curry et al., 2005). Gastrointestinal perforation, ulceration and bleeding have been associated with NSAID induced depression of normal PGE$_2$ mediated, mucosal protective mechanisms (e.g., bicarbonate and mucous secretion, epithelialization and maintenance of mucosal blood flow) (Bertolini et al., 2001).

In the present study, toxic clinical signs such as; blood in the feces, mucous mixed diarrhea and varying degrees of erosions on the mucosa of proventriculus, intestine and ceca observed in the diclofenac, aspirin, paracetamol and nimesulide groups are due to the disruption of normal integrity of gastrointestinal mucosa largely mediated by inhibition of COX1 activity. All the above said gross lesions was further evident from histological lesions such as shortening of villi, hemorrhage, hyalinization of villus epithelium of proventriculus, increased goblet cell activity, hemorrhage and desquamation and degeneration of villi of intestine.

Hemetemesis and gastric ulcerations have been reported in dogs receiving 100-300 mg/kg/day of aspirin orally for 1 to 4 weeks (Lev et al., 1972). Ulcerogenic properties of NSAIDs including diclofenac were attributed to decreased prostaglandin production in gastric fundic mucosa (Kobayashi et al., 1985).
The varying degrees of damage caused by a number of NSAIDs including diclofenac was related to the amount of unchanged or conjugated drug excreted in the bile. Additionally, gastro-toxicity was sharply influenced by the amount of drug dissolved under various pH conditions in the stomach and the intestinal toxicity appears to depend on the biliary excretion and the enterohepatic circulation of drug and its cyclo-oxygenase inhibitory activity (Beck et al., 1990).

Nimesulide at a dose of 2 mg/kg b.i.d. found to cause gastric ulcers (multiple ulcers of varying sizes and shapes with hemorrhages) in dogs upon a four day course of administration (Ramesh et al., 2001). Similarly, in the present study, the lesions found in the gastrointestinal tracts of birds administered with nimesulide are in confirmation with the findings of Ramesh et al. (2001).

Chronic oral administration of ketoprofen caused gastrointestinal upset (e.g., ulceration, bleeding, vomiting) and has been associated with decreased platelet aggregation, because it inhibits both COX1 and COX2 (Lemke et al., 2002). In the present study, the birds administered with ketoprofen did not show any signs of gastro-toxicity except for mucous mixed diarrhea, this might be due to short-term administration of ketoprofen.

Meloxicam may be associated with gastrointestinal upsets (e.g., vomiting, diarrhea and anorexia), but such problems occur rarely and are often transient (Plumb, 2002). In the present study, gastrointestinal lesions were not observed in the meloxicam received birds which might be primarily due to the selectivity of the meloxicam to COX 2 that catalyses the formation of inducible prostaglandins, which are only needed intermittently, e.g. during inflammation (Antman et al., 2005).
Renal toxicities are mainly caused by NSAIDs due to inhibition of COX1 enzyme. They include renal vasoconstriction and renal insufficiency. Renal toxicities are not reported to occur frequently in domestic animals, but patients suffering from cardiac, liver or renal diseases, hypovolemic patients and patients receiving nephrotoxic drugs are predisposed (Boothe, 2001). Diclofenac has been well documented as a nephrotoxic drug in the birds (Swetha et al., 2005; Reddy et al., 2006; Mohan et al., 2008a) and vultures (Oaks et al., 2004).

Diclofenac is a powerful inhibitor of cyclo-oxygenases and prostaglandin synthetase both of which are involved in PGE_{2} production. Diclofenac blocks PGE_{2} synthesis through inhibition of COX (Vinals et al., 1997). The swollen kidneys with prominent lobulation and tubular necrosis might be attributed to marked deposition of urates in the tubules along with degenerative changes. Similarly, diclofenac toxicity studies in chickens revealed marked tubular degeneration with lymphoid aggregates; disruption of tubular architecture with inter-tubular fibrosis and marked inter-tubular congestion with infiltration of inflammatory cells in the sections of kidney (Swetha et al., 2005; Reddy et al., 2006; Mohan et al., 2008a).

Numerous large aggregates of amorphous urate material and cell debris with infiltration of inflammatory cells accompanied with loss of normal renal architecture were prominent in kidney sections of wild juvenile *Gyps* vulture which died after experimental exposure to diclofenac toxicity (Meteyer et al., 2005). The black colored uric acid crystals observed in the kidney section of group II birds are indicative of visceral gout. Further, there are many reports in concurrence with the present findings, wherein, similar microscopic lesions were recorded in the kidneys of birds, which died
due to visceral gout (Uma et al., 1999). The extensive visceral gout has been the prominent abnormality reported in most of the postmortem reports of vultures, where the mortality was attributed to diclofenac toxicity leading to visceral gout (Oaks et al., 2004).

In avian species, the excretion of uric acid takes place at the proximal convoluted tubules and process considered to be energy-dependent (Siller, 1981; Goldstein and Skadhauge, 2000). When kidneys fail to remove the uric acid efficiently from the blood, tissues become supersaturated with uric acid resulting in urate salt precipitation as crystals (Lumeij, 1997). In the present study, deposition of black colored radiating or amorphous uric acid crystals in the kidney tubules of diclofenac administered birds are indicative of urate salt precipitation as crystals.

Necrosis of proximal convoluted tubule would compromise uric acid excretion, leading to rapid elevation of uric acid concentration in blood. Once the saturation point reached in the blood, uric acid would rapidly precipitate as crystals on the organ surface and within organ parenchyma resulting in death (Johnson, 1979). The deposition of uric acid crystals on visceral organs such as heart, liver and kidneys in group II birds could be attributed to renal failure resulting in visceral gout and death.

Renal tubular necrosis and visceral gout was also reported in king eiders and spectacled eiders treated with ketoprofen (Mulcahy et al., 2003). Also, necropsy of vultures died due to ketoprofen toxicity evidenced gross morphological changes, characterized by bilateral severe nephrotoxicity with diffuse visceral gout (Naidoo et al., 2010a). In the contrary, no gross or histological alterations in kidneys were observed in broiler chickens administered with ketoprofen, which probably indicates ketoprofen does not induce renal toxicity in broiler chickens at dose of 4 mg/kg upon oral administration.
Further, in support of this finding, no change in serum biochemical parameters such as creatinine, BUN and uric acid were observed for this group suggesting ketoprofen is comparatively safe in broiler chickens.

In a safety study conducted by Jayakumar et al. (2008) to evaluated the effect of paracetamol on kidneys of broiler chickens did not reveal any adverse effects even after repeated treatment with paracetamol (10 mg/kg, IM) for seven consecutive days. Swetha et al. (2005) did not evidence any adverse effects of meloxicam or nimesulide on kidneys of birds even after repeated administration. Reddy et al. (2006) reported absence of gross morphological or histological changes in the kidneys of birds administered with nimesulide. The present findings are in agreement with the earlier reports.

In the present study, in diclofenac treated group, there was elevated serum AST and ALT activity, decrease in serum protein, albumin and histopathological changes in liver section are suggestive of hepatic damage (Lumeij, 1999; Denman et al., 1983). Diclofenac causes a rare but potentially fatal hepatotoxicity that may be associated with the formation of reactive metabolites (benzoquinones imines) via a hepatic cytochrome P450 catalyzed oxidation which contribute to diclofenac-mediated hepatic injury (Kappus, 1986; Tang, 1999). The gross histopathological changes observed in the liver of diclofenac administered birds are similar to the earlier reports (Swetha et al., 2005; Reddy et al., 2006; Mohan et al., 2008a).

In a safety study (Mohan et al., 2008b), the effect of paracetamol on liver of avian species was studied in broiler wherein, paracetamol upon intramuscular administration for 7 days at a dose of 10 mg/kg, IM revealed hepatotoxicity which was attributed to the gradual degeneration of hepatocytes due to repeated dosing of paracetamol. It is also
reported that paracetamol in humans gets biotransformed to toxic NAPQI (N-acetyl-p-benzoquinone imine) metabolite found to cause fatal hepatic degeneration and necrosis (Burke et al. 2006). The lesions observed in liver sections of birds in paracetamol group were confirmation with the earlier findings.

The observed increased levels of AST, ALT and ALP in nimesulide group was consistent with the histological lesions of liver such as degeneration and necrosis of hepatocytes around the bile duct, hyperplasia of bile duct epithelium accompanied with massive infiltration of inflammatory cells. All the observed pathological changes might be attributed to the toxic effect exerted by nimesulide on liver.

Nimesulide is a selective cyclo-oxygenase-2 (COX-2) inhibitor, with only residual activity against cyclo-oxygenase-1 (COX-1) (Bennett, 1999) almost exclusively metabolized and cleared by the liver (Davis and Brogden, 1994). It has been reported that the drug can cause several types of liver damage, ranging from mild abnormal function such as increase in serum aminotransferase activity to severe organ injuries such as hepatocellular necrosis or intrahepatic cholestasis (McCormick et al., 1999; Schattner et al., 2000; Lucena et al., 2001). The molecular mechanisms underlying this drug induced toxicity have not yet been fully elucidated. However, experimental evidence suggests that during metabolism different reactive metabolites are produced that covalently modify proteins (Bernareggi, 1998), impose oxidative stress (Berson et al., 1991; Ritter and Giganti, 1998) and causes mitochondrial injury of hepatocytes (Mingatto et al., 2000).

In the diclofenac group, congestion, hemorrhage, sub-epicardial edema and urate deposition with infiltration of inflammatory cells observed in the section of heart are possibly due to hyperuricemic condition, which was observed biochemically through
increased uric acid levels and further confirmed histologically using DeGalanthas stain. The depositions of black stained uric acid crystals in rosette pattern with occasional focal irregular black spots were suggestive of presence of uric acid crystals in the heart. The accumulated uric acid crystals could have played a key role in damaging the cardiac muscle fibers causing focal myocarditis characterized by loss of cross striations, fragmentation of myocardial fibers leading to infiltration of inflammatory cells.

From the present study, it was concluded that, meloxicam, celecoxib and ketoprofen were safe for use in avian species compared to other NSAIDs like diclofenac, aspirin, paracetamol and nimesulide. The conclusion was based on; (a) none of the birds administered these three drugs showed classical clinical signs of toxicity except for mucous mixed diarrhea in ketoprofen group. They remained apparently healthy throughout the experimental period. (b) The majority of biochemical parameters analyzed remained unaltered indicating absence of toxic effect on vital organs. (c) There were no observable gross and histological lesions. Thus, highlights the absence of toxic effects in any of the organs or tissue sections examined. Considering these factors, ketoprofen, meloxicam and celecoxib at the dose of 4 mg/kg, 0.5 mg/kg and 3.5 mg/kg, respectively administered orally for five days did not exert any toxicity or adverse effects in broiler chickens.

In the present study, the observed toxic effects were related to the compound as such or the metabolite. Further studies are required in this direction. In addition, long term toxicity studies are also required.