II. REVIEW OF LITERATURE

Although a large number of non-steroidal anti-inflammatory drugs (NSAIDs) are being discovered following the initial breakthrough of aspirin, comparatively a very small number of NSAIDs are being used in birds unlike in other domestic animals or human beings. The literature related to toxicity of NSAIDs in birds is also scanty. Hence, to have a fair idea on the toxicity of commonly used NSAIDs, a review on all such regularly used NSAIDs in veterinary practice has been taken up.

2.1 History of NSAIDs

Salicylates present in plant extracts are clinically used from centuries. Vane (1971) defined the principal mode of action as cyclo-oxygenase inhibition. The isolation of salicylic acid in the 19th century from Willow bark led to its widespread use as an antipyretic, analgesic and anti-inflammatory agent. Since then, many non-steroidal compounds with anti-inflammatory properties have been discovered. The group, NSAIDs, is generally restricted only to those substances that act by inhibiting components of the enzyme system in the metabolism of arachidonic acid and formation of eicosanoids. These drugs may be structurally classified as carboxylic acids (R-COOH) or enolic acids (R-COH).

The carboxylic acid derivatives include the salicylates (e.g. acetylsalicylic acid), acetic acids (e.g. indomethacin), propionic acids (e.g. ibuprofen), anthranilic acids (e.g. meclofenamic acid) and aminonicotinic acids (e.g. flunixin). The enolic acids include the pyrazolones (e.g. phenylbutazone) and the oxicams (e.g. meloxicam) (Lees et al., 1991).

In addition to these, other drugs commonly known as COX2 inhibitors are derived...
from sulfonanilides (e.g. nimesulide) and diarylheterocycles, COX2 selective inhibitors (e.g. rofecoxib, celecoxib, valdecoxib, parecoxib and etoricoxib) (Antman et al., 2005).

Several NSAIDs including salicylates, are routinely used in veterinary practice in the treatment of febrile states and inflammatory disorders of various animal species, including cats, dogs, horses, chickens, swine and cattle (Yeary and Swanson, 1973; Mathur et al., 1974; Barber et al., 1974; Yeary and Brant, 1975; Fagot, 1975; Gingerich et al., 1975; Eyre et al., 1976). In recent years, the treatment of pain in animals is becoming an important ethical issue including in food producing animals. NSAIDs are necessary to manage the pain and inflammatory conditions in food producing birds (chickens, ducks, turkeys, gheese, swans, quails, guinea fowls, ostrich, emu etc), pet birds as well as in zoo birds.

2.2 Pharmacodynamics

Studies in the early 1970s by Smith and Willis (1971) and Vane (1971), separately suggested that the principal mode of action of aspirin like drugs was by the inhibition of the enzyme cyclo-oxygenase, an enzyme that converts arachidonic acid into eicosanoids.

Eicosanoids, such as prostaglandins and leukotrienes, are 20-carbon-chain derivatives of cell membrane phospholipids. These compounds are synthesized when oxygen reacts with the polyunsaturated fatty acids of cell membrane phospholipids. The most important of these fatty acids is arachidonic acid released into the cell from damaged cell membranes and thereby serves as a substrate for enzymes to generate eicosanoid products. Cyclo-oxygenases are located in all cells except in mature red blood cells, adds oxygen to arachidonic acid, generating unstable prostaglandin endoperoxides.
Subsequent peroxidase reaction results in the formation of prostaglandins and thromboxanes. Eicosanoids are potent mediators of inflammation (Boothe, 2001).

Two isoforms of cyclo-oxygenase are known to exist; cyclo-oxygenase 1 (COX1) and cyclo-oxygenase 2 (COX2). COX1 mediates the formation of constitutive prostaglandins produced by many tissues, including gastrointestinal cells, platelets, endothelial cells and renal cells. Prostaglandins generated from COX1 are constantly present and produce a variety of normal physiologic effects; protection of gastrointestinal mucosa, hemostasis and maintenance of renal blood flow. COX2 catalyses the formation of inducible prostaglandins, which are only needed intermittently, e.g. during inflammation (Antman et al., 2005).

NSAIDs appear to inhibit both COX1 and COX2. The amount of drug necessary to inhibit each of the two isoforms provides a basis for assessing the relative safety and efficacy of each drug. The ratio of COX2 to COX1 describes the amount of drug necessary to inhibit the respective isoforms of the cyclo-oxygenase enzyme in an experimental environment. A COX2/COX1 ratio of less than one is desirable, since a drug that inhibits COX2 (inducible) prostaglandins at lower concentrations than that necessary to inhibit COX1 (constitutive) prostaglandins are considered safer. Salicylates, flunixin and phenylbutazone have high COX2/COX1 ratio (preferential COX1 inhibitors) and meloxicam and carprofen are examples of NSAIDs with a favourable ratio (preferential COX2 inhibitors) (Osiri and Moreland, 1999; Boothe, 2001).

Inhibition of cyclo-oxygenase as the sole anti-inflammatory mechanism of action by NSAID’s has been disputed. These drugs seem to alter cellular and humoral immune responses and may suppress inflammatory mediators other than prostaglandins. It has
been reported that several neutrophil functions may be inhibited, depending on the drug (Boothe, 2001). For example, piroxicam inhibits both the generations of superoxide ions and release of lysosomal enzymes, whereas ibuprofen does neither (Weissmann, 1991).

Prostaglandins are involved in the modulation of pain responses in birds and because the physiologic mechanisms involving prostaglandins in birds are reported to be similar to those in mammals (Nicol, 1992). Therefore, it seems reasonable to expect that the NSAID would produce analgesia even in birds. Besides, there are also reports relating to expression of cyclo-oxygenases in the CNS of vertebrates and their relative expression varies depending on the species (Bergh and Budsberg, 2005). A broad tissue distribution and existence of COX in chickens has been delineated by Yamada et al. (2006). It has been described that prostaglandins and COXs participate in avian nociception and act peripherally and centrally similar to prostaglandins and COXs of mammals (Anhut et al., 1979; Machin et al., 2001). Galliformes and Anseriformes were the primary avian orders mainly used to evaluate pharmacokinetics and physiologic and analgesic effects of COX1 and COX2 inhibitors (McGeown et al., 1999; Baert and DeBacker, 2002; Baert and DeBacker, 2003).

2.3 Clinical pharmacology and therapeutic use

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of various musculoskeletal inflammatory diseases in animals. In veterinary medicine, they are used for the treatment of disease conditions like spondylitis, laminitis, mastitis, endotoxic shock and colic in the horse and for the control of pain associated with trauma or surgery (Boothe, 2001).
The ability of non-steroidal anti-inflammatory drugs to suppress inflammation and subsequent tissue damage is considered vital, since the inflammatory process might result in organ damage that renders the animal unprofitable or useless for production (Kopcha and Alwynelle, 1989). The suppression of pain, that causes distress to the animal considered to be an important pharmacological property of NSAIDs (Danbury et al., 1997).

Few indications are also suggested in avian species for the treatment with NSAIDs that could be beneficial (Bauck, 1990). Although, an anatomic and metabolic difference between avian species and mammals exists, the dosage regimens reported for treatment in birds are mostly extrapolated from small mammals and therefore at certain times, the extrapolation of drug dosages might lead to risk or disaster (Dorrestein, 1992).

2.3.1 Trauma

Pain management is considered to be an indispensable constituent in the practice of veterinary medicine mandated by the Institute of Laboratory Animal Resources, Washington DC, USA, in all vertebrate species. Furthermore, adequate pain control was regarded as an essential part of therapeutic intervention in acute and chronic diseases of all animals, including birds (Anon., 1996).

NSAIDs exert their anti-inflammatory effects not only on peripheral tissues but also has centrally acting anti-nociceptive effects and therefore, found to be useful when administered post-trauma and in cases of severe illness (Urquhart, 1993; Mazario et al., 1999). The beneficial effect of NSAIDs has been mentioned for ostriches in reducing the post-operative pain induced due to leg surgery (Weissmann, 1991).
Indomethacin has been shown to have anti-nociceptive effects in pigeons (Benzi et al., 1966) and anti-inflammatory effects in the chicken (Ito and Bohn, 1986). Both sodium salicylate and acetaminophen have anti-nociceptive properties in pigeons in the same order of magnitude as seen for some mammalian species (Brune et al., 1974). Ketoprofen (0.5 to 2.0 mg/kg, IM) did not establish any detectable analgesia in Mallard ducks following a skin incision (Machin, 1998).

Hocking et al. (2005) conducted experiments in domestic fowl to evaluate efficacy and optimum doses for the NSAIDs like carprofen, flunixin, ketoprofen and sodium salicylate on the pain related behavior induced by microcrystalline sodium urate arthritis. From their study, the minimum effective doses for carprofen, flunixin and ketoprofen were found to be 30, 3 and 12 mg/kg, IM, respectively and the minimum dose for sodium salicylate ranged from 100 to 200 mg/kg, IM.

The analgesic efficacy of meloxicam was studied in Hispaniolan parrots (Amazona ventralis) with experimentally induced arthritis (intra-articular injection of microcrystalline sodium urate suspension into intertarsal joint) and extent of weight bearing and rotational perch walking were used as outcome measures (Cole et al., 2009). It was concluded that, parrots treated with meloxicam at 1.0 mg/kg, IM had better return to normal (baseline) weight bearing on the arthritic pelvic limb, compared with control parrots or parrots treated with meloxicam at 0.05, 0.1 and 0.5 mg/kg, IM.

Adult wild-strain Mallard ducks exhibited decrease response to a noxious stimulus (closure of a hemostat around the right leg at the midpoint of the metatarsal bone) upon administration of ketoprofen (5 mg/kg, IM) 30 minutes prior to induction of
noxious stimulus suggesting that ketoprofen had analgesic effects in Mallard ducks (Machin and Livingston, 2002).

2.3.2 Coccidiosis

A study associated with pathogenesis of Cryptosporidium baileyi showed that treatment with indomethacin reduced the excretion of oocysts and shortened the time of excretion (Hornok et al., 1999). In broiler production, possible indications of NSAIDs are in digestive coccidial and bacterial infections and inadequate intestinal equilibrium to sustain good weight gain (Cristofol et al., 2000). In one of the trials, effects of indomethacin (non-selective COX1 and COX2 inhibitor) and nimesulide (a selective COX2 inhibitor) were studied in coccidial infection. Indomethacin and nimesulide at 50 ppm and at 400, 100 and 50 ppm, respectively were mixed with broiler starter ration. No improvement in lesion scores or infection induced suppression of weight gain were noticed, but the treatment reduced oocyst output per chick with indomethacin and with the low dose (50 ppm) nimesulide (Allen, 2000). Furthermore, an NSAID, ibuprofen, upon administration at an oral dose of 100 mg/kg showed a reduction in oocyst shedding and intestinal coccidial lesions (Vermeulen, 2002).

2.3.3 Sudden death syndrome

Use of NSAIDs had been suggested for treatment of sudden death syndrome in turkeys and heavy breed chickens. Experiments with dietary aspirin were done in both species, but no beneficial effect on sudden death syndrome could be found (Proudfoot and Hulan, 1983; Boulianne and Hunter, 1990). The etiology of broiler ascites is still under investigation by several researchers. However, a possible mechanism reported for ascites in broiler chickens was speculated to be an imbalance between oxygen supply and
the amount of oxygen required to sustain rapid growth rates and high food efficiencies (Decuypere et al., 2000). In addition, proportional changes in the respiratory and cardiovascular systems, shifts in the glycolytic: oxidative muscle metabolism ratio and structural changes within the lung capillary system are some of the structural endogenous factors described in causing broiler ascites. The other possible mechanism described was pulmonary hypertension induced ascites (Decuypere et al., 2000).

In another experiment, the effect of dietary aspirin on ascites in broiler chickens kept in a hypobaric chamber, to mimic high altitude was studied. A moderate reduction in ascites was reported in aspirin treated birds compared to control. Slow growth rate have known to reduce ascites, smaller weight recorded for these birds kept at high altitude was the reason given for the reduced incidence of ascites (Balog et al., 2000).

2.3.4 Heat stress

Acetylsalicylic acid (aspirin) is a potent antipyretic drug that has shown to lower the body temperature of heat stressed chickens. In addition, several authors have investigated a possible beneficial effect of salicylates on the growth and egg production of chickens during heat stress. An experiment in layer hens, showed improvement on growth and size of eggs upon feeding the feed containing combination of ascorbic acid (200 mg/kg) and aspirin (0.20%). However, with similar combination, no effect was observed on the body temperature (Oluyemi and Adebano, 1979).

Broiler chickens have been found to show higher survival rates after treatment with flunixin during heat stress periods (Oliver and Birrenkott, 1981). In another study, an improvement in feed intake, egg production, eggshell weight and reduction in mortality was observed in the aspirin treated (600-800 mg/kg feed) laying hens (Abd-
Ellah et al., 1997). Other researchers found no improvement in egg production at 0.2% aspirin in the feed. But, they found a significant depression in egg production at higher levels (0.6%) (Mathur et al., 1974). However, Stilborn et al. (1988) found that, dietary supplementation of aspirin provided a little benefit to heat stressed broilers.

McDaniel and Parker (2004) conducted a study with an objective of improving fertility of male broiler breeders exposed to elevated ambient temperatures, but, inclusion of acetyl salicylic acid at 0.15% in the male's diet did not decrease the body temperature when roosters were exposed to elevated ambient temperatures. In the same study, 0.15% dietary acetyl salicylic acid was found to be detrimental to fertility of heat stressed broiler breeder males.

An experiment was carried out at Sids Poultry Research Station, Egypt, to study the effect of acetyl salicylic acid on productive and reproductive performance of Bandara and Dandarawi chickens during summer season (June, July and August) where environmental temperature ranged from 22-38 °C and humidity from 35-60% (Galil, 2004). It was concluded that, supplementation of acetyl salicylic acid to layer diets at 20 ppm during summer season improved both production and reproduction performance.

2.3.5 Growth or egg production

An experiment on performance of hen and eggshell quality after chronic feeding of aspirin added diet showed no improvement in egg production or feed efficiency (McDaniel et al., 1993a). In addition, they found decreased early hen livability and eggshell quality in the aspirin fed chickens. However, an earlier study, showed good results after a 0.05% dietary aspirin treatment with increased egg production and feed efficiency (Thomas et al., 1966). Further, an experiment was carried out to study the
short term effect of high doses of aspirin in the diet of laying hens (4-7.5 g/kg feed) and observed no change in investigated parameters such as egg production, time of oviposition and behavior of birds (Gilbert et al., 1982).

A common avian reproductive dysfunction is the inability to deposit shell adequately during egg formation. To mention few defects; soft-shelled and shell-less eggs were laid prematurely. It was hypothesized that the prostaglandins may be responsible for the premature expulsion of some soft-shelled or shell-less eggs. But, it was concluded that, aspirin could only reduce the production of shell-less eggs in favor of the soft-shelled eggs. Probably, due to physiological limitations in calcium mobilization, shell-less eggs might not have converted into hard-shelled eggs (Balog and Hester, 1991). Also, the fertility and hatchability of eggs exposed to elevated incubation temperatures was investigated with or without administration of aspirin to layer breeders. No difference in hatchability of eggs was found (McDaniel et al., 1993b).

A study was performed by Urick et al. (2009) to determine the effects of dietary aspirin treatment on incidence of ovarian cancer and progression in the hen. A standard layer diet containing 0.1% aspirin were fed to hens for a period of one year. Aspirin treated hens showed tendency for inhibition of progression of ovarian cancer and egg production. Further, their experiment showed that, treatment with aspirin could be utilized to identify hens with early stages of the ovarian cancer.

2.3.6 Locomotion disturbances

Degeneration of the hip and other joints was reported in male breeding turkeys at the end of their breeding life and also evidenced that the birds affected with hip and other joints experienced pain during locomotion (Hocking, 1988; Duncan et al., 1991). These
findings suggested that existence of locomotion disturbances in birds is a possible indication for using NSAIDs.

In a study performed by Hughes and Sufka (1990) revealed naproxen administration attenuated inflammation and algesia (measure of foot withdrawal latency to a thermal stimulus) in chickens. Aspirin was used in duck production for tenosynovitis and in turkey industry for certain leg problems (Jouglar and Benard, 1992). Treatment with carprofen has proven to increase the walking speed of affected chickens, providing evidence that birds with moderate lameness suffer pain when they walk (McGeown et al., 1999). Danbury et al. (1997) have proven that lame broiler chickens self select more feed containing an analgesic agent than sound birds. Later, an experimental inflammation protocol was developed by Hocking et al. (1997) to determine the effectiveness of different analgesics for reducing articular pain in domestic fowl.

Arthritis was induced by sodium urate in the hock joint and behavioral profiles were studied. Bupivacaine at a dose of 3 mg was able to restore behavioral profiles of the birds. Different opioid agonists, however, with a high affinity for the µ receptor, could not alter the pain behavior after the inflammatory stimulus (Gentle et al., 1999).

Broiler breeder males with leg lesions walked more slowly when they were given an injection of naloxone, an opioid antagonist. This was the first evidence for the existence of an endogenous system of analgesia in the fowl. It was demonstrated that, a powerful system of endogenous analgesia operates in the chicken in response to specific environmental stimuli (Gentle and Corr, 1995) and it was considered that, the possible incentive of food or a sexual encounter might stimulate this endogenous analgesia in birds with leg lesions. The sodium urate induced arthritis model was used in chickens to
study the effects of changes in attention on pain coping behavior and inflammation. The study suggested, the changes in attention not only reduce the pain of arthritis, but also, the peripheral inflammation, since less elevated skin temperature was evinced over the inflamed joint (Gentle and Tilston, 1999).

2.3.7 Pain related to beak trimming

Another possible use of NSAIDs could be in the analgesic therapy of beak-trimmed chickens. It is known that the beak trimming excites nociceptors in the beak leading to short-term acute pain and a reduction in feed intake (Glatz et al., 1992; Hughes and Gentle, 1995).

Already experiments with a combination of bupivacaine and dimethyl sulfoxide have shown that application of this mixture on the wound can maintain the feed intake on the first day after trimming. This indicated that some of the acute pain had been relieved (Glatz et al., 1992).

Additionally, topical application of phenylbutazone to the beaks of chickens resulted in maintenance of feed intake during the first 24 h after beak trimming, compared with a decrease in intake among chickens that were not treated (Glatz et al., 1992).

2.4 Toxicities of NSAIDs

The majority of adverse reactions reflect the inhibitory effects of NSAIDs on prostaglandin activity. In most of the NSAID toxicity, the gastro-intestinal, hematopoietic and renal systems are affected. Miscellaneous effects associated with use of NSAIDs include hepato-toxicity, aseptic meningitis, diarrhoea and central nervous system depression (Boothe, 2001).
2.4.1 Hematological alterations

Larson (1963) reported that the cats, which consumed 81-130 mg/kg of aspirin for 12 days, suffered from anemia and suppression of bone marrow. Aspirin brought about inhibition of platelet aggregation and alleviated some systemic changes in cattle seen in acute systemic anaphylaxis (Eyre and Burka, 1979). The anti-thrombotic actions of aspirin previously demonstrated in man and laboratory animals were established in the horse by Cambridge et al. (1991).

It was shown that flunixin had a potent inhibitory effect on the thromboxane B₂ (TXB₂) generation by platelets in blood (McKellar et al., 1989).

All NSAIDs are able to impair platelet activity by impairing thromboxane synthesis. 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).

Higgins et al. (1980) reported that phenylbutazone inhibited leukocyte migration. High doses of phenylbutazone were reported to have a free radical scavenging activity that could affect the release of lysosomal enzymes (Schuster et al., 1985).

Phenylbutazone has been shown to inhibit PGE₂ and PGI₂ concentrations in the inflammatory exudates and TXB₂ generation in the blood platelets of the horses (Lees et al., 1987). Phenylbutazone, in dogs and rats along with monkeys showed much greater enterohepatic circulation and it has been reported that the drug causes hypoproteinemia in the monkeys without affecting the albumin vs. globulin ratio (Heywood and James, 1980). Phenylbutazone has also been implicated in the fatal blood dyscrasia in dogs
although the incidence of such adverse reactions was likely to be very low (Watson et al., 1980).

In the horse, gastrointestinal ulceration with concomitant protein loosing enteropathy was reported with phenylbutazone therapy (Snow et al., 1981). Toxicity to phenylbutazone is well recognized in man and aplastic anemia has been said to account for the deaths of over 1000 people in 20 years (Brooks et al., 1986).

Certain studies have suggested that prostaglandins mediate pathology of subarachnoid hemorrhage and that reduced synthesis of prostacyclin in the vessel wall might contribute to cerebral vasospasm. Piroxicam was found to be more efficacious in preventing both vascular and behavioral changes in the experimental models of the cerebral vasospasm (White and Robertson, 1983).

Galbraith and McKellar (1991) also reported that piroxicam inhibits the generation of TXB₂ in the blood of the dogs by more than 90% following intravenous administration at a dose of 0.3 mg/kg. Meloxicam was observed to be a potent inhibitor of TXB₂, 6-ketoPGF₂ and bicyclic PGE₂ in inflammatory exudate in an equine model of acute inflammation (Lee et al., 1991).

### 2.4.2 Serum biochemical alterations

Some of the non-steroidal anti-inflammatory drugs like piroxicam, indomethacin, phenylbutazone and aspirin were used as rodenticides at certain parts of Nigeria and were evaluated for its toxicity that included the changes in the serum biochemical components and haematological parameters in rats. Indomethacin at 5 mg/kg, piroxicam at 15 mg/kg, aspirin at 20 mg/kg and phenylbutazone at 10 mg/kg dosed to each rat in the group orally using a stomach cannula for fourteen days. Among these NSAIDs, indomethacin and
piroxicam increased the total bilirubin level and decreased blood urea nitrogen. Aspirin, indomethacin and phenylbutazone increased serum AST levels compared to the control group. In addition, indomethacin caused increase in levels of serum ALT. The increase in serum levels of AST and ALT were associated with increased liver damage in rats possibly due to adverse effects of NSAIDs (Abatan et al., 2006).

In a study (Swan et al., 2006a), diclofenac sodium (0.8 mg/kg) administered by oral gavage to two species of vulture *Gyps africanus* and *Gyps fulvus* revealed increase plasma concentrations of uric acid and ALT. Similarly, an increase in serum uric acid, creatinine, blood urea nitrogen, ALT and AST concentration were reported in the chickens administered with diclofenac (2.5 mg/kg) (Swetha et al., 2005; Reddy et al., 2006; Mohan et al., 2008a). The reported biochemical alterations are possible indications of damage caused by NSAID diclofenac to vital organs such as kidney and liver.

Swetha et al. (2005) reported no significant change in serum biochemical parameters (uric acid, creatinine, BUN, ALT and AST) of broiler chickens administered with other NSAIDs meloxicam (0.5 mg/kg) and nimesulide (2 mg/kg). Similarly, no change in serum biochemical parameters like creatinine, cholesterol, ALP and AST were reported in nimesulide (2 mg/kg or 5 mg/kg) treated birds (Reddy et al., 2006).

Mohan et al. (2008a) did not observe any variation in serum biochemical parameters (uric acid, creatinine, BUN, ALT and AST) in the birds after repeated intramuscular administration of ketoprofen (4 mg/kg). On the contrary, safety study conducted by Naidoo et al. (2010a), reported an increase in serum uric acid and ALT concentrations in vultures administered with a single oral dose of ketoprofen (5 mg/kg).
Gradual and significant increase in serum ALT and AST concentration in the chickens administered with acetaminophen (10 mg/kg, IM for seven days) was reported by Mohan et al. (2008b).

2.4.3 Gastrointestinal toxicity

Gastrointestinal erosions and ulcerations are the most common and serious side effects of NSAIDs reported frequently. Inhibition of prostaglandin E$_2$-mediated bicarbonate and mucus secretion and decreased blood flow may be responsible in combination. Direct irritation of acidic drugs may be important and impaired platelet function may also contribute to mucosal bleeding (Boothe, 2001).

Gregerson (1916) reported positive chemical tests for blood in the feces of patients with rheumatic fever receiving salicylates. Hurst and Lintott (1939) reported gastric bleeding in a person after taking aspirin. Larson (1963) reported gastric lesions in cats, which consumed 81-130 mg/kg of aspirin for 12 days.

Hematemesis and gastric ulcerations have been reported in dogs receiving 100-300 mg/kg/day of aspirin orally for 1-4 weeks (Lev et al., 1972). Aspirin caused renal papillary necrosis in rats and was probably responsible for the analgesic nephropathy linked to the administration of aspirin in man (Wiseman, 1975).

Intragastric aspirin at doses of 30 or 100 mg/kg caused decrease in pyloric sphincter pressure, increase of duodeno-gastric reflux and changed mucosal potential difference in dogs (Pantoja et al., 1979).

Gastrointestinal motor disturbances have also been attributed to high doses of aspirin. Lysine aspirin was said to reduce the frequency of reticular contractions, reduced abomasal motility and cyclical pattern of intestinal motility (Honde and Bueno, 1984).
The gastric lesions in dogs after administration of 25 mg/kg of aspirin were characterized. The lesions were confined to the distal fundic and pyloro-antral regions of stomach, the lesions varied from mild petechial to linear hemorrhages (Lipowitz et al., 1986). Similar type of lesions were also observed in the case of sodium restricted anaesthetized dogs (Zambroski et al., 1988).

The common cause of ulcers in small animals were due to the administration of NSAIDs, many of which healed as soon as the therapy was discontinued. Buffered aspirin might be somewhat less irritating than plain aspirin. Enteric coated aspirin tablets to dogs was discouraged because systemic absorption from these tablets was unpredictable (Papich, 1993).

In a separate study, the effectiveness of misoprostol (3 μg/kg, PO t. i. d.), a synthetic prostaglandin E₁ analog, in preventing aspirin (35 mg/kg, PO t. i. d.) induced gastroduodenal injury were studied in adult mixed breed dogs. From their findings, it was concluded that, misoprostol effectively decreased endoscopic detectable mucosal lesions in dogs induced by aspirin (Johnston et al., 1994).

Flunixin meglumine appeared to be less toxic than phenylbutazone but foals given 1.1 mg/kg/day, IM for 30 days developed signs of toxicosis (Traub-Dargatz et al., 1988). In another study, a dose of 6.6 mg/kg/day intravenously for 5 days was necessary to produce clinical signs of toxicosis in a group of foals (Carrick, 1989).

Flunixin meglumine was reported to induce gastrointestinal lesions in dogs when administered at therapeutic dosages. In a study conducted to find out the effects of flunixin on the gastrointestinal tract of dogs, the degree of mucosal damage appeared to
be dose dependent with pyloric mucosal lesions appearing sooner in dogs when given at higher doses (Steven et al., 1990).

Adams et al. (1969) found that, a dose of 8 mg/kg of naproxen in dogs when administered orally produced no clinical signs of toxicity over a 30 day period but at post mortem revealed gastrointestinal ulcers.

Gastrointestinal tract bleeding, weakness, anorexia and abdominal pain, haemetemesis and melena were observed in a case of naproxen intoxication in the dog (Felkai, 1983). Clinical cases of ibuprofen poisoning in dogs were reported with signs of renal failure, vomiting and melena (Spyridakis et al., 1986).

Gastrointestinal ulceration was the most likely side-effect of carprofen toxicity, although in the rat it has much wider safety ratio (ulcerogenic dose/anti-inflammatory dose) than either indomethacin or aspirin (Randell and Baruth, 1976). It was found that, in the dogs, a 9 mg/kg of aspirin administered orally for 14 days was well tolerated without any significant change in mean serum protein concentration, which would be expected in an animal with gastrointestinal lesion and protein losing enteropathy (McKellar et al., 1991).

The acute and chronic toxicity of the phenylbutazone was investigated in dogs (Hazleton et al., 1953). Phenylbutazon produced gastrointestinal erosions and ulcerations in dogs (Kirsner and Ford, 1955).

Gastrointestinal side effects of NSAIDs are common. Indeed, as improbable that effective therapeutic concentrations can be achieved with most drugs without some degree of damage and that it was not necessarily prevented by the parenteral use of the NSAIDs. The gastrointestinal mucosa can still be exposed to drugs through enterohepatic
circulation of drugs. This was true with the case of piroxicam toxicity (Galbraith and McKellar, 1991).

Ulcerogenic properties of NSAIDs including diclofenac were attributed to decreasing prostaglandin production in gastric fundic mucosa (Kobayashi et al., 1985).

The degree of damage caused by a number of NSAIDs including diclofenac was related to the amount of unchanged or conjugated drug excreted in the bile and the drug solubility appeared to be not a contributing factor to the toxicity. It was concluded that, in rat, it was caused by different mechanisms, gastro-toxicity was sharply influenced by the amount of drug dissolved under the 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).

Anti-inflammatory efficacy and gastrointestinal irritancy; a comparative one month repeat oral dose studies in the rat with nabumetone, ibuprofen and diclofenac showed that repeated oral dosing of nabumetone for one month maintained anti-inflammatory efficacy but diclofenac and ibuprofen caused gastrointestinal irritancy as evidenced by the mucosal damage and blood loss (Melarange et al., 1991).

Nimesulide did not produce stress induced gastric lesions even at 30 times the anti-inflammatory dose. This supported the hypothesis that inhibition of COX1 caused unwanted side effects (Nakatsugi et al., 1996).

All NSAIDs do prevent colon cancer but cause adverse side effects. Nimesulide with fewer side-effects has been thought to be an useful candidate for suppression of the colon cancer (Nakatsugi et al., 1997).
COX1 makes an important contribution to inflammatory responses. To achieve desirable anti-inflammatory effects, nimesulide had to be given at doses, at which selectivity was lost, leading to suppression of the gastric prostaglandin formation and subsequent mucosal damage (Wallace et al., 1998).

Nimesulide at a dose of 2 mg/kg, PO, 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).

Administration of diclofenac sodium at a dose of 3mg/kg, PO, b. i. d. for a period of four days reported severe punched out ulcers in gastrointestinal tract of calves (Shridhar and Narayana, 2007).

Combining non-steroidal therapies (commonly referred to as ‘stacking’) do not reduce the potential for toxicity. Gastrointestinal ulceration and protein losing enteropathy were reported when a combination of phenylbutazone and flunixin meglumine was utilized, even though each drug was administered at the published and seemingly appropriate dose (Reed et al., 2006).

Non-steroidal anti-inflammatory drugs (NSAIDs) are well recognized for causing 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, therefore inactivation of the cyclo-oxygenase enzymes by NSAIDs leads to a decrease in prostaglandin production, which in turn, impairs mucosal blood flow and leads to mucosal injury and inflammation (Boothe, 2001).
Gastrointestinal bleeding secondary to NSAID usage is a known adverse effect in mammals. Nevertheless, in avian species such adverse effect is not reported. In support of the previous statement, Cole et al. (2009) reported negative results for fecal occult blood test for all Hispaniolan parrots (Amazona ventralis) following administration of meloxicam at 0.05, 0.1 and 0.5 mg/kg (IM, q 12 h, 3 times) indicating possible absence of this adverse effect in parrots.

2.4.4 Nephrotoxicity

Renal toxicity is mainly caused by NSAIDs due to inhibition of COX1 enzyme. They include renal vasoconstriction and renal insufficiency. Renal toxicity is not reported to occur frequently in domestic animals with NSAIDs, but patients suffering from cardiac, liver or renal diseases, hypovolemic patients and patients receiving nephrotoxic drugs are predisposed (Boothe, 2001). Nephrotoxicity is one of the important adverse effect of NSAIDs reported very frequently (Ng et al., 2008). Two case reports of flurbiprofen toxicity have been reported in dogs with signs of renal failure evident from increased blood urea nitrogen and creatinine (England, 1987; Marlow, 1987).

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).

Renal insufficiency associated with usage of NSAIDs in birds was reported by (Meteyer et al., 2005). Further, the pathology and pathophysiology of diclofenac toxicity have also been described in vultures that were naturally poisoned or experimentally exposed to diclofenac (Oaks et al., 2004; Meteyer et al., 2005; Swan et al., 2006a). Visceral gout was one of the prominent abnormalities reported in most of the postmortem reports of vultures (Oaks et al., 2004; Meteyer et al., 2005; Swan et al., 2006b) and in
chickens that died due to diclofenac toxicity (Swetha et al., 2005; Reddy et al., 2006, Mohan et al., 2008a). The visceral gout is a disorder of metabolism that allows uric acid to accumulate in the tissues (Chen and Schumacher, 2008). Uric acid is the end product of purine metabolism and is produced normally by the body during tissue remodeling and breakdown (Moyer and John, 2003). Historically, gout is known as "the disease of kings" or "rich man's disease". The cause of gout in birds was reported an account of renal failure and considered to be analogous to hyperuricemia in humans (Arun and Azeez, 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). Therefore, decreased ATP levels either by hypoxia or direct cytotoxicity, would contribute to hyperuricemia. Moreover, 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. The causes of gout described are of two major categories; increased synthesis of uric acid and decreased clearance of uric acid by the kidneys. High intake of purine rich foods such as red meat and poultry meat (especially the offal foods like liver, kidney, heart, tripe etc.) are thought to be an important causative factor that enhances uric acid synthesis in the body (Arun and Azeez, 2004).

The cellular reaction associated to uric acid crystal formation and deposition resulting in gout. Thus, the formed crystals are considered less soluble under acidic conditions and any condition predisposing to acidosis further precipitates urate crystals (Halabe and Sperling, 1994). These urate crystals at certain situations are speculated to
stimulate phagocytosis by neutrophils and initiate the inflammatory cascade reaction. Interleukin-1 and tumour necrosis factor-α are known to be involved in the inflammatory cascade (Arun and Azeez, 2004).

Julian (1982) described progressive obstruction of the ureters which resulted in ascending renal disease in severe cases with the microscopic changes in the kidneys secondary to urate deposition in the ureters and distal ducts.

Further, studies related to histological examination of kidney sections of vultures died due to diclofenac toxicity had revealed extensive renal pathology displaying universal necrosis of proximal convoluted tubules accompanied with uric acid crystals in tubules which are possible indications of failure of kidneys to remove uric acid efficiently from the blood (Meteyer et al., 2005). The possible hypothesis proposed by Meteyer et al. (2005) for diclofenac induced preferential necrosis of the proximal convoluted tubules was related to the high metabolic activity of renal tubular cells leading to more hypoxia compared to the cells in distal or collecting tubules that are less metabolically active (Brown, 1985).

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 comparison to the healthy kidney sections of control group (Swetha et al., 2005; Reddy et al., 2006; Mohan et al., 2008a).

Renal tubular necrosis and visceral gout was also reported in king eiders and spectacled eiders treated with ketoprofen (Mulcahy et al., 2003). Further, a study with flunixin in quails also reported to have induced glomerular pathology (Klein et al., 1994).
In another study (Naidoo et al., 2010a), necropsy of vultures died due to ketoprofen toxicity evidenced gross morphological changes characterized by bilateral severe nephrotoxicity with diffuse visceral gout. All these changes were reported to be identical to those found in vultures *G. bengalensis, G. afrianus, G. fulvus* and *G. coprotheres* died after treatment with diclofenac (Oaks et al., 2004; Swan et al., 2006a; Naidoo et al., 2009). Similar findings (gout and/or kidney damage) were also recorded in mortality cases reported in raptors following treatment with the NSAIDs flunixin and carprofen (Cuthbert et al., 2006). These studies suggest that a common mechanism of toxicity may be responsible for NSAID related nephro-toxicity across different orders of birds.

No gross or histological alterations in kidneys were reported in broiler chickens even after repeated administration with ketoprofen (4 mg/kg, IM for 7 days) (Mohan et al., 2008a). However, histological examination of kidney sections of vultures administered with single dose of ketoprofen (5 mg/kg, PO) revealed marked disruption in cellular architecture owing to the presence of a large amount of urate tophi. In addition, presence of urate spicules was also reported in many tubular lumen and were associated with necrosis of adjacent tubular cells Naidoo et al., (2010a).

In a safety study conducted by Jayakumar et al. (2008) to evaluate the effect of acetaminophen on kidneys of broiler chickens did not reveal any adverse effects even after repeated treatment with acetaminophen (10 mg/kg, IM) for seven consecutive days. Similarly, 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)
also reported absence of gross morphological or histological changes in the kidneys of birds administered with nimesulide.

### 2.4.5 Hepatotoxicity

Hepatotoxicity and deaths have been reported with per animal doses of 125-1000 mg paracetamol (Leyland and Omeara, 1974; Finco et al., 1975; Davis, 1985). The basis for toxicity was found to be partly due to the action of p-aminophenols in converting hemoglobin to methemoglobin resulting in functional anaemia and cyanosis. An additional side-effect reported was hepatotoxicity, which was attributed to the formation of its highly reactive metabolite (McKellar et al., 1991).

Further, in a safety study (Mohan et al., 2008b), the effect of paracetamol on liver of avian species was studied in broiler chickens. In their study, 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 studies (Swetha et al., 2005; Reddy et al., 2006; Mohan et al., 2008a) related to histological examination of liver of diclofenac administered birds showed necrosis of hepatocytes, sinusoidal and central vein congestion accompanied with bile duct epithelial hyperplasia.

Oral administration of indomethacin at 5mg/kg for 14 days produced periportal hepatic necrosis and kupffer cell proliferation (Abatan et al., 2006), signs simulating
acute hepatotoxicity (Klaassen, 2001). Based on their findings, it was inferred that, indomethacin does possess potential toxic effect on the liver of rats on repeated dosing.

The findings of Mohan et al. (2008a) revealed no gross or histological changes in liver of broiler chickens administered with ketoprofen (4 mg/kg, IM) repeatedly for seven days. On the contrary, gross examination of vultures died because of ketoprofen toxicity showed severe hepatotoxicity accompanied with diffuse visceral gout (Naidoo et al. 2010a).

2.4.6 Mortality

The detrimental effects of non-steroidal agents are typically dose-dependent as reported in horses receiving higher than recommended doses often over many days (Collins and Tyler, 1985). The toxic dose of phenylbutazone in a healthy horse has been reported to be 8-10 mg/kg for several days and doses of 15 mg/kg or greater, when given for multiple days were found to be lethal, with death occurring as early as day four of treatment (MacKay et al., 1983).

The early reports related to decline in the populations of three species of South Asian vultures (G. bengalensis, G. indicus and G. tenuirostris) were attributed to scavenging of dead domesticated animals which were previously treated with diclofenac (Oaks et al., 2004; Green et al., 2004).

In a safety study of diclofenac, there was high mortality in vultures G. africanus and G. bengalensis upon gavaging single dose of diclofenac (0.25, 0.8 and 2.5 mg/kg) or feeding tissues containing diclofenac (0.007 to 0.940 mg/kg) from cattle which had been treated with a course of diclofenac prior to death (Swan et al. 2006a). Contrary to these findings, in the same safety study, oral administration of meloxicam to vultures did not
cause any mortality in African white-backed vulture (*G. africanus*), *G. bengalensis* and *G. indicus* or in *G. africanus*, respectively, upon single dose of meloxicam (0.5, 1 and 2 mg/kg) and by gavaging or feeding tissues containing meloxicam (0.03 and 1.98 mg/kg) from cattle which had been treated with a higher than standard course of meloxicam prior to death. From their study, it was inferred that NSAID diclofenac induced high mortality in vultures whereas meloxicam was safe.

Further, high mortality was also reported in the broiler chickens administered with diclofenac (2.5 mg/kg, IM) (Swetha *et al*., 2005; 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). Forty per cent mortality was recorded for diclofenac (5 mg/kg) treated group and no mortality was found for nimesulide treated (2 and 5 mg/kg) group. Thus, indicating NSAIDs nimesulide and meloxicam are safe compared to diclofenac.

In another study, mortality was also reported in large sea ducks (king eiders and spectacled eiders) treated with ketoprofen but the mortality in king eiders was found to be greater at lower doses than in spectacled eiders (Mulcahy *et al*., 2003).

Safety of ketoprofen to vultures was studied using broiler chickens as an experimental model, wherein ketoprofen (4 mg/kg) on repeated intramuscular administration to broiler chicken for seven consecutive days did not induce mortality Mohan *et al.* (2008a). Where as, Naido *et al.* (2010a & 2010b) reported mortality in vultures at two different doses (1.5 mg/kg and 5 mg/kg, PO) of ketoprofen the former dose was however lesser than earlier worker (Mohan *et al*., 2008a).
Naido et al. (2010a) conducted safety evaluation of ketoprofen on captive non-released Cape griffon vultures (*G. coprotheres*) and wild-caught African white-backed vultures (*G. africanus*), both the species on the basis of earlier reports described to be susceptible to diclofenac (Swan et al., 2006a). Mortalities in both the species of vultures were reported at two dose levels (1.5 and 5 mg/kg) which were dosed with ketoprofen at dose levels ranging from 0.5 to 5 mg/kg by oral gavage or through feeding tissues from cattle dosed with ketoprofen at 6 mg/kg before slaughter (Naido et al., 2010a). But, vultures treated with 0.5-1.4 mg/kg of ketoprofen survived. It was deduced, that ketoprofen induces toxicity in these two species of vultures at doses that birds could encounter in the wild upon feeding cattle carcasses, which were treated with diclofenac before death.

Yet in another safety study (Naidoo et al., 2010b), Cape Griffon vultures (*G. coprotheres*) dosed orally with ketoprofen at a single dose of 1 mg/kg caused no toxicity. However, mortality was noticed in vultures upon administration of single higher oral dose of ketoprofen (5 mg/kg). It was also stated that birds showed clinical signs of toxicity; like depression, loss of appetite and apparent coma followed by death within 48 h of ketoprofen (5 mg/kg) administration.

In addition, combinations of NSAIDs (diclofenac sodium, 2.5mg/kg and paracetamol, 10mg/kg) upon oral administration for five days have caused mortality in broiler chickens (Mohan et al., 2009).