STUDIES OF KETOPROFEN TOXICITY IN AVIAN SPECIES

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Abstract

Certain environmental factors are very important to stabilize the wild life; avian diseases, vulture crises and ecological annoyance. This should be fixed scientifically to minimize the health hazards. Thus; we have aimed this project to evaluate effects of toxic dosage levels of Ketoprofen in broiler chickens. Two hundred and twenty five (225) healthy broiler chickens were reared upto 28 days and divided into five groups 25 birds in each group. On day 29th four groups were medicated twice a day at dose rate of 50 mg/kg body weight respectively intra-muscularly for four days. Feed and water were provided *ad libitum*. A physical examination, toxicity and mortality rate were recorded daily. Blood samples was drawn to determination the serum values of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Uric Acid, Alkaline phosphatase (ALP), and Creatinine. Postmortem performed on day 41 after all samples taken. In second experiment other 100 birds were divided into five groups comprising of 20 birds in each group. One of the groups was injected I/M Ketoprofen 5mg/kg twice a day. Postmortem performed after medication on 5th day. Based on the necropsy findings and biochemical analysis it was found that Ketoprofen was not safe drug in the avian species. Keeping in view the environmental problem (vultures crises) it is recommended that Ketoprofen which has good pharmacological effects in human medicine should be avoided in veterinary practice.

Keywords: Ketoprofen toxicity, Broiler birds, LFT's

INTRODUCTION

Reverend Edmund Stone described the efficacy of bark of the willow in the cure of agues (Fever). Stone reasoned that it would probably possess curative properties. The bitter glycoside called salicin (an active ingredient in the willow bark) was isolated in a pure form in 1829 by Leroux, who also demonstrated its antipyretic effect. On hydrolysis, salicin yields glucose and salicylic alcohol. The latter can be converted into salicylic acid either in vivo or by chemical manipulation.

Ketoprofen (Benzenacetic acid, 3-benzoyl-∝-methyl-, Orudis; Wyeth-Ayerst) shares many therapeutic activities along with unwanted effects; blood loss, intestinal or gastric ulceration and anemia. Patients taking Ketoprofen chronically have about three times higher relative risk for sever adverse gastro intestinal incidences compared to nonusers (Gabriel *et al*., 1991). The clinical usefulness of Ketoprofen is restricted by a number of adverse effects include diarrhea, tinnitus, headache with indomethacin and upper abdominal pain with Ketoprofen. The ranking of NSAIDs according to toxicity shows indomethacin, tolmetin, meclofenamate and Ketoprofen to be the most toxic with coated or buffered aspirin and ibuprofen the least.

Ketoprofen is usually absorbed rapidly after oral administration with maximal concentrations in plasma within 1 to 2 hours and the rate of absorption is reduced by food. It extensively binds to the plasma protein (99%) with about two hours half life in plasma; may slightly extend in elderly subjects. It is also conjugated with glucoronic acid in the liver and excreted via urine. Patients with renal impaired eliminate the drug more slowly.

Ketoprofen used for the treatment of excruciating pain due to acute episode of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Postoperative and posttraumatic pain, non articular rheumatism (acute painful shoulder, tendinitis, bursitis, tenosynovitis), renal colic and biliary colic, postpartum pain, cancer pain, acute attacks of gout, sciatica.

Therefore; we have aimed this project to study the toxicity of Ketoprofen in avian species, evaluate the safety in avian species and recommend the substitute generic and potency of Ketoprofen being not hazardous to wild life.

MATERIALS AND METHODS

The experimental work was conducted at experimental sheds of Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore. A total of 225, day old broiler chicks collected from the "Pakistan Hatchery" were vaccinated according to the

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vaccination schedule given in Table 1. The Ketoprofen (Profenid; Aventis Pharma) was injected IM 5mg/ Kg body weight twice a day and obtained from Orient labs. Pvt. Limited.

Table 1. Vaccination schedule

Age	Vaccine	Route of Vaccination
6th day	Newcastle disease	Eye drop
15th day	Gumboro (I.B.D)	Drinking water
21st day	Newcastle disease	Drinking water
25th day	Gumboro (I.B.D)	Drinking water

Experimental Design

On 28th day 75 birds were divided into 2 groups randomly; Group A with 50 and B with 25 birds. On 29th day Ketoprofen I/M 5mg/kg body weight injected IM for twice a day upto four days individually to each bird of group A, for four consecutive days. Group B was kept as control. The remaining 75 birds were divided into two groups C with 75 and D with 25 birds. Each bird of group C was injected Ketoprofen I/M 5mg/kg body weight injected for twice a day upto four days and group D was kept as control group and no medication given to them. Findings are have been summarized Table 5.

Feed and water was provided *ad libitum.* A daily basis record of physical examination, sign and symptoms and toxicity was maintained regularly.

Sample Schedule and Parameters Determined

The detail of sampling schedule and parameters determined were also published by Asif et al (2009). We have collected 3 ml blood sample from birds of each group (A, B, C and D) before starting the medication on 29th day. Then blood sample from the same birds were drawn from wing vein (vena cutanea ulnaris) on days 33, 37 and 41 after medication for determination of serum values of following parameters; Aspartate Transaminase (Thomas, 1998), Alanine Transaminase, Uric acid, Alkaline phosphatase (ALP) (Bessey et al., 1946), Concentration of creatinine in serum (Tietz, 1986).

Clinical Findings and Statistical Analysis

The clinical findings mortality and postmortem was recorded during experimental period. The collected data were analyzed statistically with one way analysis of variance (Steel et al., 1982). On days 41st and 47th postmortem were done. Three parameters including postmortem, liver, kidney biopsies and stainings were examined.

RESULTS

The biochemical parameters; uric acid, creatinine, alanine transaminase, aspartate transaminase, alkaline phosphatase of were noted for test and control birds before and after Ketoprofen. The results of necropsy findings of experimental chicks in different groups were also recorded as given below.

Biochemical Parameters of Ketoprofen**:**

Each bird of group B was injected I/M Ketoprofen 5 mg/kg body weight for twice a day up to four days. Following parameters were measured.

Uric Acid: As shown in table 2 the mean values of uric acid of Ketoprofen was 5.806380 mg/dl, 6.026060 mg/dl, 5.975420 mg/dl and 6.913780 mg/dl at before medication, 1st day after medication, 5th and 9th day after medication respectively.

There was no significant difference in the mean values of uric acid of Ketoprofen table 3.

Creatinine: The mean values of creatinine in Ketoprofen group was 1.188800 mg/dl, 1.117026 mg/dl, 1.220550 mg/dl and 0.991480 mg/dl at before medication, 1st day after medication, 5th and 9th day after medication respectively Table 2.

There was no significant difference in the mean values of creatinine in Ketoprofen group table 3.

Table 2. Various biochemical parameters of Ketoprofen group n=5 values given as MEAN± SEM

Uric Acid mg/dl	Creatinine mg/dl	ALT µ/L	AST µ/L	ALP µ/L	Time of sample collection
5.806380 ± .545235	1.188800 ± 1.904E-02	9.965180 ± .563780	177.30200 ± 6.428437	28.316000 ±1.121742	Before medication
6.026060 ±8.215E-02	1.117026 ± 1.541E-02	10.777700 ± 817404	250.69920 ±14.638531	53.39000 ±1.538587	1st day after medication
5.975420 ± .420863	1.220550 ± .135490	10.417980 ± .422810	210.36320 ±9.467424	58.65600 ±2.733095	5th day after medication
6.913780 ± .489202	0.991480 ± 3.897E-02	10.844120 ± .523152	230.44000 ±27.582283	50.12600 ±1.654538	9th day after medication

Alanine Transaminase (ALT): As shown in table 2 the mean values of Alanine transaminase of Ketoprofen was 9.965180 µ/L, 10.777700 µ/L, 10.417980 µ/L and 10.844120 µ/L before medication, 1st day after medication, 5th and 9th day after medication respectively. There was significant difference in the mean values of alanine transaminase of Ketoprofen table 3.

Table 3. ANOVA of Ketoprofen

		Sum of Squares	Df	Mean Square	F	Sig.
Uric acid	Between groups	3.718	3	1.239	1.376	.286
	Within groups	14.409	16	.901		
	Total	18.127	19			
Creatinine	Between groups	.155	3	5.169E-02	2.019	.152
	Within groups	.410	16	2.560E-02		
	Total	.565	19			
ALT	Between groups	2.441	3	.814	.453	.719
	Within groups	28.769	16	1.798		
	Total	31.211	19			
AST	Between groups	14680.431	3	4893.477	3.540	.039
	Within groups	22120.517	16	1382.532		
	Total	36800.948	19			
ALP	Between groups	2670.053	3	890.018	51.473	.000
	Within groups	276.657	16	17.291		
	Total	2946.710	19			

Table 4. Various biochemical parameters of normal group n=5 values given as mean± SEM

Uric Acid mg/dl	Creatinine mg/dl	ALT µ/L	AST µ/L	ALP µ/L	Time of sample collection
5.031800 ±.209874	1.052080 ±3.172E-02	10.149740 ±.687521	193.55568 ±2.584983	27.252000 ±1.187440	0 day
4.776720 ±.474997	0.972669 ±6.390E-02	10.205000 ±.376771	199.43520 ±18.580837	34.470000 ±3.520395	1st day
5.479140 ±.400607	1.134440 ±9.002E-02	10.269660 ±.401202	158.98660 ±16.616974	33.548000 ±3.204323	5th day
4.874880 ±.152974	1.066040 ±9.043E-02	10.351780 ±.507856	166.81000 ±10.338517	27.824400 ±1.302525	9th day

Table 5. ANOVA of normal

		Sum of Squares	Df	Mean Square	F	Sig.
Uric acid	Between groups	1.447	3	.482	.851	.486
	Within groups	9.071	16	.567		
	Total	10.519	19			
Creatinine	Between groups	6.607E-02	3	2.202E-02	.824	.499
	Within groups	.427	16	2.671E-02		
	Total	.493	19			
ALT	Between groups	.113	3	3.780E-02	.029	.993
	Within groups	20.670	16	1.292		
	Total	20.784	19			
AST	Between groups	5883.275	3	1961.092	2.135	.136
	Within groups	14698.768	16	918.673		
	Total	20582.043	19			
ALP	Between groups	212.301	3	70.767	2.197	.128
	Within groups	515.349	16	32.209		
	Total	727.650	19			

Table 6. Result of necropsy findings of various drugs in broilers chicks

Drug	Dose	Postmortem lesions		
		Site of injection	Liver	Kidney
Phenylbutazone	50 mg/kg n = 15	10/15	7/15	0/15
	100 mg/kg n = 15	15/15	12/15	0/15
Ketoprofen	5 mg/kg n = 15	0/15	5/15	0/15
	10 mg/kg n = 15	9/15	8/15	0/15
Dipyron	50 mg/kg n = 15	0/15	3/15	0/15
	100 mg/kg n = 15	4/15	6/15	0/15
Piroxicam	1 mg/kg n = 15	0/15	0/15	0/15
	2 mg/kg n = 15	0/15	3/15	0/15
Control	No medication n = 15	0/15	0/15	0/15
	No medication n = 15	0/15	0/15	0/15

Aspartate Transaminase: As shown in table 2 the mean values of aspartate transaminase of Ketoprofen was 177.30200 µ/L, 250.69920 µ/L, 210.36320 µ/L and 230.44000 µ/L at before medication, 1st day after medication, 5th and 9th day after medication of Ketoprofen.

There was significant difference in the mean values of aspartate transaminase of Ketoprofen table 3.

Alkaline Phosphatase (ALP): As shown in table 2 the mean values of alkaline phosphatase of Ketoprofen was 28.316000 µ/L, 53.390000 µ/L, 58.65600 µ/L and 50.126000 µ/L at before medication, 1st day after medication, 5th and 9th day after medication of Ketoprofen respectively.

There was significant difference in the mean values of alkaline phosphatase of Ketoprofen table 3.

Results of necropsy findings of experimental chicks in different groups.

The birds were slaughtered at the end of experiment and different lesions in kidney, liver and muscles were recorded.

Each bird of group B was injected I/M Ketoprofen 5 mg/kg body weight for twice a day up to four days.

Each bird of group L was injected I/M Ketoprofen 10 mg/kg body weight for twice a day up to four days. Findings are mentioned in table-6.

Control group: Group E and O was kept control no medication given to them and group 0 was kept control no medication given to them. Findings are mentioned in table-6.

DISCUSSION

This drug is mainly used in human practice the toxic effects reported are gastrointestinal discomfort, gastric pain, nausea vomiting diarrhea constipation headache and vertigo.

On necropsy it was observed that liver was damaged and there was no lesions observed in kidney. Necrosis at the injection site was recorded in most of the birds in this group. There was no significant rise in the serum levels of uric acid and creatinine which indicated that Ketoprofen had no toxic effects at the kidney. The findings of present study are in agreement with the observations of (MacAllister *et al.*, 1937) who reported that there were no renal lesions developed in horses treated with Ketoprofen, (Swinkels *et . al.*, 1994) studied that Ketoprofen had no gastric and renal side effects in pigs. These observations support the results of this research.

Garcia (1998) and Alarcon *et.al* in (2002) reported the risk of gastrointestinal bleeding and or perforation in Ketoprofen therapy. This situation was not observed during this study however there was increase in serum levels of ALT, AST and ALP showing hepatotoxicity. The postmortem of the control group revealed no

abnormalities particularly in liver, kidneys and muscles. Similarly there was no significant difference in the serum values of uric acid and creatinine ALT, AST and ALP in the samples collected at the different times during experiments in the group.

MacAllister *et al.* (1973) reported adverse affect of Ketoprofen. There were 16 horses assigned randomly to receive physiological saline 10 ml solution or Ketoprofen (2.2 mg/kg of body weight) every 8 hours, for 12 days. All the horses were killed on 13th day to obtain the complete post mortem report. Mean CBC values were noticed within normal limits for all groups. Moreover; the postmortem examination showed the severely Ketoprofen affect at the glandular portion of the stomach and gastrointestinal tract. Horses treated with the saline and Ketoprofen did not develop renal lesions. Under the conditions of this study and with total daily doses that exceeded the manufacturers' recommended doses, the toxic potential of the Ketoprofen in clinically normal adult horses.

Semrad (1993) reported the Ketoprofen efficacy for the therapy of endotoxemia in neonatal calves. Ketoprofen (Ketofen, 2.2 mg/kg, i.v.), and ketorolac tromethanine (Toradol, 1.1 mg/kg, i.v) each ameliorated the clinical signs of endotoxaemia and LPS-induced lacticaemia, but failed to significantly alter the degree of leucopenia or hypoglycemia associated with infusion of LPS.

Swinkels *et al*. (1994) reported the antipyretic effect on Ketoprofen 3 mg/kg and flunixin 2 mg/kg in pigs. These drugs were administered intramuscular 8 and 32 hours for endobronchial challenge with pleura-pneumonia (noticed at postmortem examination). Ketoprofen showed a highly significant antipyretic effect. The reduction of the food consumption in Ketoprofen treated pigs was significantly less than infected or non-medicated controls.

Landoni, *et al*. (1996) reported the effects of the flunixin, tolfenamic acid, S (+) Ketoprofen (KTP) and R (-) Ketoprofen on the production of beta-glucuronidase (beta-glu), interleukin-6) IL-6), prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF alpha)and interleukin-1 (IL-1) induced by lipopolysaccharide (LPS) stimulated equine synoviocytes.

Beltran *et al.*, (1998) reported the D (dextro) Ketoprofen as an active E (enantiomer) Ketoprofen form of the racemic compound. It is a new drug belonging to arylpropionate family of non-steroidal anti-inflammatory group. They compared the efficacy and safety of dexKetoprofen trometamol with the equivalent dose of enantiomers this drug in a randomized, double-blind multicenter, 3-week trial of adult patients with osteoarthritis pain at knee. The patients were then randomly assigned to receive either D-Ketoprofen trometamol 25 mg tid (N-89) or E-Ketoprofen 50 mg tid (N-94) after 7-15 days washout period. After 3 weeks; at the end of treatment, the main outcome showed the significantly better in the D-Ketoprofen trometamol group than in the Ketoprofen group. Moreover; physician assessment pointed out 75% of the D-Ketoprofen patients had improved better. The results showed D-Ketoprofen trometamol is more effective than Ketoprofen in short-term symptomatic therapy; suggesting the tolerability of D-Ketoprofen trometamol as favorable treatment than Ketoprofen. Thus, substitution of D-Ketoprofen for racemic Ketoprofen is potentially less hazardous in clinical practice.

Alarcon *et al.* (2002) reported the changes in cyclooxygenase expression, prostaglandin synthesis, gastric toxicity and glutathione metabolism of Ketoprofen. They express the response to damage in rats in form of the generation of oxygen free radical and neutrophil infiltration. Result showed Ketoprofen dose-dependently toxicity. Ketoprofen was also less ulcerogenic. Bonina *et al.*, (2002) also reported the *in vitro* polyoxyethylene esters of naproxen (2a-e), Ketoprofen (1a-e) and diclofenac (3a-e) to evaluate their solidity in simulated gastric fluid (pH 2.0 buffer) and at pH 7.4 phosphate buffer. They also studied the susceptibility to enzymatic cleavage in human plasma. The results obtained indicated that all tested pro-drugs exhibited good analgesic effect and the esters have low irritating effect on the gastric mucosa than respective parent drug.

CONCLUSION

In conclusion, there was no mortality recorded in any group of broiler chicks given Ketoprofen. Based on the necropsy findings and biochemical analysis it was also found that ketoprofen was not safest drug in the avian species. Keeping in view the environmental problem (vultures crises) it is recommended that ketoprofen might having good pharmacological outcome in human but should be avoided in veterinary practice.

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Received: 15-03-2011
Accepted: 06-04-2011