Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 11	No. 4	pp. 649-652	2012

Original Research Paper

Haemato-Biochemical Studies on Diclofenac, Ibuprofen and Nimesulide Induced Toxicity in Broilers

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 4-5-2012
Accepted: 8-5-2012

Key Words:
Haemato-biochemical studies
NSAIDS
Toxicity
Broilers

ABSTRACT

The present experimental work was conducted to elucidate the haemato-biochemical studies in broiler chicks when treated with diclofenac sodium, ibuprofen and nimesulide. For this study 120 apparently healthy broiler chicks of either sex were randomly divided into 10 groups comprising 10 birds in 9 groups and 30 birds in control group. The broilers of diclofenac groups were administered 10, 20 and 30 mg/kg body weight of diclofenac sodium for 5 days. Similarly the broilers of ibuprofen groups were administered with 15, 30 and 45 mg/kg body weight of ibuprofen with feed for 5 days. The birds of nimesulide groups were administered with 10, 20 and 30 mg/kg nimesulide for 5 days. The birds of control group were maintained on feed and plain water to serve as control. There was no significant difference in haematological parameters in birds which are intoxicated with diclofenac, ibuprofen and nimesulide. On the other hand there was significant difference in alkaline phosphatase and BUN biochemical parameters, but no significant difference in total protein and calcium intoxication in chicks.

INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce pain in various diseases and postoperative conditions. NSAIDS have three major functions *viz.*, anti-inflammatory, antipyretic and analgesic. Their anti-inflammatory effect is mainly due to their ability to inhibit the activities of cyclo-oxygenase enzymes (COX-1 & COX-2) that mediate the production of prostaglandins from arachidonic acid. The enzyme COX is responsible for normal physiological turnover of prostaglandin. It maintains the gastric lining integrity and renal homeostasis .

The primary mechanism of diclofenac, responsible for its anti-inflammatory, antipyretic and analgesic action, is inhibition of prostaglandin synthesis by inhibition of cyclo-oxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis. Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side-effect of diclofenac. Diclofenac was found to be causing pathological changes in kidneys of the vultures, which ultimately lead to the gout (Oaks & Khan 2004).

Ibuprofen inhibits both COX-1 and COX-2. It appears that its analgesic, antipyretic and anti-inflammatory activities are achieved principally through COX-2 inhibition whereas COX-1 inhibition is responsible for its unwanted effects on platelet aggregation and the GI mucosa.

Nimesulide is a preferential COX-2 inhibitor and therefore, assumed to be safer, in clinical use. Its gastrointestinal tolerance has not been proven to be superior to other NSAIDs because various epidemiological studies give little weight to the hypothesis that selective inhibition of COX-2 may have a sparing effect on the GI tract.

Gyps vulture population across the Indian subcontinent are declining rapidly and evidence indicates that veterinary use of the nonésteroidal anti-inflammatory drugs (NSAIDs), especially diclofenac is the major cause of this hazard (Taggart et al. 2007).

Keeping the above facts in view, the investigation has been designed to study the haemato-biochemical changes in broilers which are intoxicated with diclofenac, nimesulide and ibuprofen.

MATERIALS AND METHODS

Experimental chicks: A total number of 120 broiler chicks were procured form the market. All the chicks were already vaccinated against Marek's disease prior to the delivery. On the first day of procurement the chicks were given Electrol in water in the forenoon. From the afternoon the chicks received starter mash.

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

Experimental groups: The chicks were divided into 4 major groups and within each major group they were divided

Table 1: Experimental and control groups of broilers in the experiment.

Groups	Drug Name	Dose & Duration (5 days)	Dose & Duration (5 days)	Dose & Duration (5 days)
Control C	No Drug	No drug	No Drug	No Drug
D (Treatment)	Diclofenac	(D1) 10mg/kg b.wt	(D2) 20mg/kg b.wt	(D3) 30mg/kg b.wt
B (Treatment)	Ibuprofen	(B1) 15 mg/kg b.wt	(B2) 30mg/kg b.wt	(B3) 45mg/kg b.wt
N (Treatment)	Nimesulide	(N1) 10 mg/kg b.wt	(N2) 20mg/kg b.wt	(N3) 30mg/kg b.wt

into 3 sub groups having 10 chicks in each sub group. Experiment was carried out with 120 chicks (Table 1).

1. After 5th day of every treatment the blood samples were collected from wing vein of birds with the help of sterile needle for biochemical and haematological studies.
2. The blood samples were collected with anticoagulant EDTA@2mg/mL.
3. The blood samples were also collected without anticoagulant and serum was collected and stored at temperature (-10 to -20ºC) for biochemical studies.

Hematological studies: For determination of different haematological values, blood was collected from each bird. EDTA was used as anticoagulant at all the occasions. The birds from each group were used for haematological study. The methods of Jain (1986) and Campbell (1988) were used for haematological studies. The haematological parameters studied include:

1. Total erythrocyte count
2. Total leucocyte count
3. Differential leucocytic count
4. Packed cell volume
5. Haemoglobin

Biochemical studies: For all the biochemical estimations blood was collected from wing vein. For biochemical estimation broiler chicks were taken on at random basis from each group and blood was collected in clean, dry and sterile test tube.

The biochemical parameters include:

1. Alkaline phosphatase
2. Total protein
3. Calcium
4. Blood urea nitrogen

Statistical analysis: Analysis of variance and critical difference test were carried out by the formulae of one way CRD.

RESULTS AND DISCUSSION

Haematology: As per the results (Tables 2, 3) there is no significant difference in the haematological parameters of the treated groups. Similarly, Takahashe et al. (2002) did not observe any significant haematological changes in monkeys after oral administration of diclofenac sodium @1mg/kg b.wt. Anonymous (1999) reported that there may be the diclofenac toxicity including anaemia in baboon after the oral administration of diclofenac @50mg/kg body weight. Shridar & Narayanan (2007) reported that the prolonged use of diclofenac sodium is toxic to proliferating haemopoietic tissue leading to the development of significant anaemia in the birds. Anonymous (2004) also reported the anaemia in beagle dogs after the oral administration of diclofenac @3 mg/kg body weight. There may be the increase in the MCV due to the diclofenac toxicity which release large size immature RBCs in circulation from bone marrow leading to the macrocytic anaemia. The findings are contradictory to the observation in dogs with no haematological changes observed as a result of oral administration of diclofenac sodium @ 0.03, 0.1 and 0.3 mg/kg b.wt by oral route(Anonymous 2003). In the present study no such significant changes were observed in haematological parameters in broilers.

Sero-Biochemical Studies.

Total protein: There was no significant difference in the level of serum protein due to all the three NSAID's toxicities in the present study (Table 4). Contradictory reports are available with respect to total plasma proteins during NSAID toxicities in birds. The observations of hypoproteinaemia due to diclofenac sodium toxicity in this study confirms the finding of the diclofenac toxicity in rabbits (Sakr et al. 1996). The oral administration of nimesulide may also cause hypoproteinaemia in treated nimesulide groups. Similar results have been reported by Dunn(2000) in birds.

Baert (1994) reported that the nimesulide, a specific Cox-2 inhibitor, may cause decrease in serum protein level in birds. The increase in the level of total proteins was not due to accelerated catabolism of blood proteins. This clearly indicates that NSAIDs have direct effect on protein synthesizing units of hepatocytes which in turn is reflected in low level of total protein in serum. The present finding also corroborates the work of Divis & Brogden (1994).

Alkaline phosphatase: The results on the changes in alkaline

Table 2: Mean values of Hb, PCV and TEC in control and treated groups.

Name ofgroups	Hb (gm/dl)	PCV (%)	TEC (million/mm^3)
N1	8.76 ± 0.37	30.16 ± 0.65	3.00 ± 0.36
N2	8.78 ± 0.36	33.5 ± 1.17	2.83 ± 0.40
N3	8.93 ± 0.44	32.16 ± 1.13	3.00 ± 0.36
B1	9.01 ± 0.39	32.00 ± 0.85	2.66 ± 0.33
B2	9.38 ± 0.22	31.16 ± 1.01	3.00 ± 0.36
B3	8.78 ± 0.33	31.83 ± 0.87	2.66 ± 0.33
D1	8.48 ± 0.29	31.5 ± 0.88	2.66 ± 0.33
D2	8.41 ± 0.33	31.33 ± 1.25	2.83 ± 0.30
D3	9.13 ± 0.38	31.66 ± 1.22	2.83 ± 0.40
Control	8.96 ± 0.36	32.5 ± 1.11	2.33 ± 0.21

No significant difference between the groups.

Table 3: Mean values of TLC and DLC in control and treated groups.

Name of groups	TLC (thousand/mm^3)	Heterophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
N1	24.50 ± 1.94	23.66 ± 1.60	62.83 ± 1.70	8.83 ± 0.40	2.16 ± 0.30	2.50 ± 0.22
N2	26.50 ± 0.88	24.33 ± 1.11	62.83 ± 1.24	8.50 ± 0.61	2.00 ± 0.36	2.33 ± 0.21
N3	26.33 ± 2.01	21.16 ± 1.49	67.16 ± 1.79	7.66 ± 0.55	1.83 ± 0.30	2.16 ± 0.30
B1	24.16 ± 1.86	23.66 ± 1.20	64.00 ± 1.84	8.50 ± 0.61	1.83 ± 0.40	2.00 ± 0.25
B2	25.00 ± 1.82	23.16 ± 1.32	64.50 ± 1.54	8.00 ± 0.57	2.00 ± 0.36	2.33 ± 0.33
B3	25.83 ± 1.92	22.33 ± 1.35	65.66 ± 1.96	7.50 ± 0.61	2.33 ± 0.33	2.16 ± 0.30
D1	23.83 ± 1.79	20.33 ± 1.30	64.83 ± 1.92	9.00 ± 0.51	2.00 ± 0.25	2.16 ± 0.30
D2	24.00 ± 1.06	20.83 ± 1.40	68.00 ± 1.41	8.00 ± 0.57	2.33 ± 0.33	2.00 ± 0.25
D3	27.66 ± 1.60	22.50 ± 1.30	64.83 ± 1.74	8.33 ± 0.55	2.00 ± 0.36	2.33 ± 0.21
Control	23.83 ± 1.32	21.66 ± 1.22	65.33 ± 1.78	8.50 ± 0.61	2.16 ± 0.30	2.33 ± 0.21

No significant difference between the groups.

Table 4: Mean values of BUN and total protein in control and treated groups.

Name of groups	BUN (mg/ 100 mL)	Total Protein (gm/dl)
N1	1.91^B ± 0.24	4.88 ± 0.31
N2	1.81^B ± 0.52	4.98 ± 0.29
N3	1.55^B ± 0.28	5.06 ± 0.23
B1	2.36^A ± 0.52	4.81 ± 0.37
B2	2.95^A ± 0.37	4.83 ± 0.28
B3	3.41^A ± 0.19	5.01 ± 0.27
D1	3.66^A ± 0.01	4.78 ± 0.33
D2	3.66^A ± 0.03	4.75 ± 0.33
D3	3.73^A ± 0.02	5.00 ± 0.26
Control	1.61^B ± 0.22	4.58 ± 0.23

Capital letters indicate significant difference between control and treatment groups.

Table 5: Mean values of alkaline phosphates and calcium in control and treatment groups.

Name of groups	Alkaline phosphates (IU/L)	Calcium (mg/100mL)
N1	171.66^B ± 0.66	10.03 ± 0.45
N2	172.50^B ± 0.76	10.06 ± 0.30
N3	173.00^A ± 0.51	10.11 ± 0.50
B1	171.50^B ± 0.61	10.41 ± 0.37
B2	173.33^B ± 0.54	10.51 ± 0.52
B3	171.83^B ± 0.80	10.56 ± 0.49
D1	171.50^B ± 0.60	10.76 ± 0.37
D2	172.83^B ± 0.74	09.76 ± 0.23
D3	171.83^B ± 0.74	10.58 ± 0.52
Control	170.83^B ± 0.83	09.81 ± 0.38

Capital letters indicate significant difference between control and treatment groups.

phosphatase in broilers due to NSAIDS toxicity are given in Table 5. The mean values in the present study of exposure of broiler chicks to NSAIDs may cause significant elevation of serum alkaline phosphates. The increase in the alkaline phosphatase may be due to an increase in osteoblastic activity, an impairment of liver function and an obstruction in the bile flow. There was no indication of excess osteoblastic activity at the lamellar surfaces as well as the costochondral junctions. On the contrary microscopic examination of liver revealed vacuolar degeneration of hepatocytes and proliferation of bile duct. Therefore, elevation of serum alkaline phosphatase in the present study might be due to hepatotoxic effect of the NSAIDs. In contrast, Shah et al. (1994) reported elevation of serum alkaline phosphatase in rabbits after the oral administration of

ibuprofen. Similar effects were also be reported by Radino (1987) after their oral administration as nimesulide in goats.

BUN: The results on the changes in BUN in broilers due to NSAIDS toxicity are given in Table 4. There might be increase in the BUN level in the birds which have been treated with the diclofenac. Toxicity has been reported in rabbits (Sakr et al. 1996), mice (Hickey et al. 2001), mongrel dogs (Ramesh et al. 2002) and crossbred calves (Shridar & Narayanana 2007). Similar effect was also reported by Mendret (2006) after the oral administration of ibuprofen in rabbits. Lambert et al. (2006) also reported elevation of BUN after the oral administration of nimesulide in dogs. The observations like hyperuricaemia in the toxic groups clearly reflected that repeated use of NSAIDs at therapeutic level for prolonged period may induce marked renal dysfunction. This fact has been fully substantiated by pathological observations of the kidneys i.e., congestion and degeneration of Bowman's capsule, cloudy swelling to hydropic degeneration with accumulation of hyaline mass in tubular lumens leading to formation of necked tubules with or without lesions of interstitial nephritis in this study.

Calcium: In the present study there is no significant change in the serum calcium level in all the NSAID intoxicated groups of broiler chicks (Table 5). Similar observation have also been made by Prakash (1999) after the oral administration of diclofenac in birds. On the other hand Dunn (2000) reported the increase in the calcium level in poultry after the administration of ibuprofen. This may be due to the increased activity of the bone cells.

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