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Haemato-biochemical and Immunological Study on NSAIDs Induced Acute Toxicity in Broiler Chicken

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ABSTRACT

The present study was aimed to elucidate the effect of Non steroidal anti-inflammatory drugs (NSAIDs) induced toxicity in the broiler chicken. For this purpose, 120 broiler chicks were utilized and divided into 4 major groups (C, D, B and N) and within each major group the chicks were divided into 3 sub groups having 10 chicks each. The control chicks received normal diet without any medicine. Diclofenac was used @ 10, 20 and 30 mg/kg b.wt. in D1, D2 and D3 group respectively. Ibuprofen was fed @ 15, 30, 45 mg /kg b.wt in B1, B2 and B3 group respectively. The nimesulide group viz. N1, N2 and N3 were given Nimesulide @ 10, 20 and 30 mg/kg b.wt respectively. NSAIDs induced toxicity showed no effect on the haematological parameters of broiler chicks in the present study, however, there was increase in the serum alkaline phosphatase level in intoxicated birds indicating hepatotoxicity compared to the control group with highest value of 173.33 ± 0.80 IU/L in B3. Further, atrophy of spleen and bursa of fabricius was observed in intoxicated birds. Highest atrophy of spleen (1.1 gm/kg body weight) was observed in N3 and D3 while as maximum bursal atrophy of 1.20 ± 0.06 gm/kg body weight was recorded in B3.

Keywords: Broilers, haematology, serum characteristics, lymphoid organs

Non steroidal anti-inflammatory drugs (NSAIDs) have the ability to inhibit cyclo-oxygenase enzymes, which are involved in the formation of prostaglandins. However, there are marked differences between drugs in their selective inhibition of the two subtypes of cyclo-oxygenase, COX-1 and COX-2, the latter being involved with the modulation of inflammation-mediated responses and pain, while the former modulates blood flow to the kidneys. Further, the ability of NSAIDs to inhibit both these subtypes has been implicated as a cause of the side effects occasionally associated with the use of some NSAIDs (Jain *et al.,* 2009). Gyps vulture populations across the Indian subcontinent are declining rapidly and evidence indicates that veterinary use of the NSAIDs especially Diclofenac is the major cause (Oaks *et al*., 2004a). Exposure of vultures to diclofenac is likely to arise from the consumption of livestock carcasses that have been treated shortly before death and high residual value of Diclofenac was found in the body tissue of carcass (Taggart *et al.,* 2007). Diclofenac was found to be causing pathological changes in the kidneys of the vultures, which ultimately lead to the gout (Oaks and Khan, 2004). Very little literature is available on the NSAIDs induced toxicity in broiler chicken, therefore, the present study was designed to elucidate the effect of various NSAIDs viz. Diclofenac, Ibuprofen and Nimesulide in broiler chicken.

MATERIALS AND METHODS

A total number of 120 day old broiler chicks were utilized in the present study. All the chicks were vaccinated against Marek's disease prior to the procurement. The birds were

Table 1. Details of different treatment groups and dose rates of drugs used

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

Table 2. Effect of different doses of Diclofenac, Ibuprofen and Nimesulide on TEC, PCV and Hb in broiler chicken

Group	TEC million/mm3	PCV %	Hb gm/dl
Control	**2.33 ± 0.21**	**32.50 ±1.11**	**8.96 ± 0.36**
N1	3.0 ± 0.36	30.16 ± 0.65	8.76 ± 0.37
N2	2.83 ± 0.40	33.50 ± 1.17	8.78 ± 0.36
N3	3.0 ± 0.36	32.16 ± 1.13	8.93 ± 0.44
B1	2.66 ± 0.33	32.0 ± 0.85	9.01 ± 0.39
B2	3.0 ± 0.36	31.16 ± 1.01	9.38 ± 0.22
B3	2.66 ± 0.33	31.83 ± 0.87	8.78 ± 0.33
D1	2.66 ± 0.33	31.5 ± 0.88	8.48 ± 0.29
D2	2.83 ± 0.30	31.33 ± 1.25	8.41 ± 0.33
D3	2.83 ± 0.40	31.66 ± 1.22	9.13 ± 0.38

There was no significant difference within the groups

Table 3. Effect of different doses of Diclofenac, Ibuprofen and Nimesulide on TLC and DLC in broiler chicken

Group	TLC Thousand/mm3	Heterophils%	Lymphocyte%	Monocytes %	Eosinophils %	Basophils %
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
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 ± 1.4	68.00 ± 1.41	8.00 ± 0.57	2.33 ± 0.33	2.00 ± 0.25
D3	27.66± 1.60	22.50 ± 1.3	64.83 ± 1.74	8.33 ± 0.55	2.00 ± 0.36	2.33 ± 0.21
B1	24.16 ± 1.86	23.66 ± 1.2	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
N1	24.50 ± 1.94	23.66 ± 1.60	62.83 ± 1.70	8.83 ± 0.4	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

There was no significant difference within the groups

Table 4. Effect of different doses of Diclofenac ,Ibuprofen and Nimesulide on BUN and Total Protein in broiler chicken

Group	Total Protein (gm/dl)	Alkaline Phosphates (IU/L)	Calcium mg / 100ml	BUN (mg / 100ml)
Control	4.58 ±0.23	170.83^{a} ±0.83	9.81 ± 0.38	1.61 ±0.26
D1	4.78 ± 0.33	171.50 ±0.6	10.76 ± 0.37	3.66^{b} ±0.01
D2	4.75 ± 0.33	172.83 ±0.74	9.76 ± 0.23	3.66^{bc} ± 0.03
D3	5.00 ± 0.26	171.83 ±0.74	10.58 ± 0.52	3.73^{b}±0.02
B1	4.81 ± 0.37	171.50^{ab} ±0.61	10.41 ± 0.37	2.36^{b} ± 0.52
B2	4.83 ± 0.28	171.83^{ab} ±0.80	10.51 ± 0.52	2.95^{bc}± 0.37
B3	5.01± 0.27	173.33^{b} ±0.80	10.56 ± 0.49	3.41^{c} ± 0.19
N1	4.88 ± 0.31	171.66^{ab} ±0.66	10.03 ± 0.45	1.91±0.17
N2	4.98 ± 0.29	172.50^{ab} ±0.76	10.06 ± 0.30	1.81 ±0.21
N3	5.06 ± 0.23	173^{b} ±0.51	10.11 ± 0.50	1.55 ± 0.28

Means within the row with different superscripts are statistically different

Table 5. Effect of different doses of Diclofenac, Ibuprofen and Nimesulide on relative weight of Spleen, Bursa, Thymus and Caecal Tonsils in broiler chicken

Group	Spleen g/kg	Bursa of Fabricius g/kg	Thymus g/kg	Ceacal Tonsils g/kg
Control	2.5^{b} ± 0.13	2.3^{b} ± 0.17	2.5^{b} ± 0.3	2.5^{b} ± 1.14
D 1	2.30^{b} ± 0.18	2.20^{b} ± 0.06	3.40^{a} ± 2.19	3.50^{a} ± 0.25
D2	1.40^{a} ± 0.19	1.60^{a} ± 0.26	3.10^{b} ± 3.96	2.60^{b} ± 0.31
D3	1.10^{a} ± 0.05	1.30^{a} ± 0.14	2.60^{b} ± 0.28	2.5^{a} ± 0.05
B1	2.60^{b} ± 0.24	2.2^{b} ± 0.05	3.40^{a} ± 0.25	3.30^{a} ± 0.28
B2	1.50^{a} ± 0.24	1.4^{a} ± 0.19	2.80^{b} ± 0.56	2.80^{b} ± 0.3
B3	1.40^{a} ± 0.21	1.2^{a} ± 0.06	2.50^{b} ± 0.26	2.3^{a} ± 0.3
N1	1.80^{a} ± 0.31	2.0^{b} ± 0.29	3.3^{a} ± 0.25	3.40^{a} ± 0.29
N2	1.70^{a} ± 0.18	1.8^{a} ± 0.24	2.6^{b} ± 0.64	3.10^{b} ± 0.52
N3	1.10^{a} ± 0.03	1.3^{a} ± 0.16	2.4^{b} ± 0.24	2.80^{b} ± 0.85

Means within the row with different superscripts are statistically different.

housed in the deep litter system and a floor space of 2 square feet was provided to each chick. Each group had a separate marking with wire mesh. Thoroughly dried and disinfected chopped paddy straw was used as bedding for the chicks. The chicks were divided into 4 major groups (C, D, B and N) and within each major group the chicks were divided into 3 sub groups having 10 chicks each Table 1). The control chicks received normal starter feed without any medicine. Diclofenac was used @ 10, 20 and 30 mg/kg b. wt. in D1, D2 and D3 group respectively. Similarly, Ibuprofen was fed @ 15, 30, 45 mg /kg b.wt. to the birds of ibuprofen group (B1, B2 and B3). Likewise, the birds of nimesulide group (N1, N2 and N3) received 10, 20 and 30 mg/kg b.wt. Nimesulide respectively in their feed. The NSAIDs were fed to all the treatment groups for 5 days. All the chicks irrespectively of their groups were maintained on the same type of chick starter mash. The chicks were fed in the first week @ of 10g /chick but from second week onwards *ad libitum* feeding practice was adopted. All the chicks were vaccinated with F1

(Lasota) vaccination against Ranikhet disease on 6th day of age. The birds of each group had a separate feeding and watering trays. Each chick was critically inspected every day for the appearance of clinical symptoms of toxicity if any at every stage of the experiment. Appearance of clinical symptoms was carefully recorded. The mortality rate in each group was also recorded every day until the end of the experiment which lasted for 35 days.

After 5th day of treatment period, the blood samples were collected from wing vein of birds with the help of sterile needles. The blood samples were treated with anti coagulant EDTA @ 2 mg/ml and used for hematological study by using standard kits. The haematological parameters studied include total erythrocyte count, packed cell volume, hemoglobin, total leucocyte count and differential leucocytic count. For biochemical study, the samples were collected without anti coagulant and serum was obtained and stored in sterile tubes at -10 to -20ºC until further used. The biochemical parameters were included total protein, alkaline phosphatase, calcium and blood urea nitrogen (BUN). Total protein was estimated following the modified Biurate and Doumas method (Doumas and Wastsen, 1971). Alkaline phospatase was determined as per the method recommended by Kind and King (1954). For estimation of serum calcium, Trinder's method was adapted (Trinder, 1960). Blood urea nitrogen was estimated following the Diacetylmonoxime DAM method. The diagnostic reagent kit (M/S Span, India) was used for determination of urea in serum. Further, the relative weight of lymphoid organs viz. spleen, thymus, caecal tonsils and bursa of fabricius was also calculated. These organs were excised from birds of each group, weighed and the relative weights were calculated as described by Garg *et al.* (2004). For statistical analysis, Analysis of variance and critical difference tests were calculated by the formulae of one way CRD (Box *et al.*, 1978).

RESULTS AND DISCUSSION

Birds showed the observerable clinical signs like anorexia, emaciation, dehydration, depression, feather picking, swollen and painful joints after the oral administration of Diclofenac. These findings are more or less in agreement with the report made by other workers in various strains of poultry (Mishra and Singh, 1981). The birds of Ibuprofen group showed signs of dehydration, anorexia, loss of body weight, decrease in feeding, increase in thirst and even death of some broiler chicks. Similar clinical signs were observed in the birds belonging to the Nimesulide group. Mori *et al.* (2000) were also reported above clinical signs in rats after the oral administration of Nimesulide.

Hematological observations

The mean values of total erythrocyte count (TEC), packed cell volume (PCV) and haemaglobin (Hb) are presented in Table 2. There were no statistical differences in the mean values of TEC, PCV and Hb among different treatment groups when compared to the control group. Regarding TEC, the mean value of control group was 2.33±0.21 million/mm^3. Among treated groups, highest TEC of 3.00±0.36 million/mm^3 were observed in N1, N3 and B2 and lowest value of 2.66±0.33 million/mm^3 was recorded in B1, B3 and D1. Highest PCV of 33.50±1.17% was recorded in N2 and lowest value of 30.16 ± 0.65% in N1. With respect to Hb, the mean value of the control group was 8.96±0.36 gm/dl with highest value of 9.38 ± 0.22 gm/dl in B2 and lowest value of 8.41 ± 0.33 gm/dl in D2. As per the results obtained, there was no significant difference in the hematological observations of the treated groups compared to the control. These results are in accordance with Takahashe *et al.* (2002) who did not observe any significant haematogical change in monkeys after oral administration of diclofenac sodium @1 /mg/kg b.wt. However, the results are in contradiction with the reports of Shridar and Narayanan (2007) who reported that the prolonged use of diclofenac sodium is toxic to proliferating hemopoietic tissue leading to the development of significant anaemia in poultry. Further, no significant changes were observed in the total leucocyte count and differential leucocyte count in the experimental birds of present study (table.3). The mean value of TLC in control group was 23.83±1.32 thousand/mm^3. Among treatment groups, highest value of 27.66 ±1.60 thousand/mm^3 was noticed in D3 and a lowest value of were 23.83±1.79 thousand/mm^3 was achieved in D1. Regarding DLC, highest value of 24.33 ± 1.11% was observed in N2 and lowest value of 20.33 ±1.30% was observed in D1. The highest mean value of lymphocytes was 68.00 ±1.41% in D2. Regarding monocytes, highest and lowest values of 9.00 ± 0.51% and 7.50 ±0.61% were found in D1 and B3 respectively.

Biochemical observations

The mean values of serum total protein, alkaline phosphatase, calcium and blood urea nitrogen (BUN) are presented in Table 4. The highest total serum protein value of 5.06 ± 0.23 gm/dl was noticed in N3. Further, the statistical analysis did not show any significant difference in the mean values of total protein within the different groups. There was no significant difference in the level of serum protein as a result of NSAIDs toxicity in the present study. Contradictory reports are available with respect to total plasma proteins during NSAID toxicities in birds. Diclofenac sodium toxicity has been reported to induce hypoproteinemia in rabbits (Sakr *et al.,* 1996). Further, Dunn (2002) reported that the oral adminstration of Nemuslide may cause hypoproteinemia in Nemuslide treated birds. However, no such results were observed in the present study, which could be attributed to the difference in the dosage level of drugs and duration of time used.

The mean value of alkaline phosphatase was highest (173.33 ± 0.80 IU/L) in B3. There was a definite statistical increase in the level of alkaline phosphate in all the intoxicated groups when compared to the control. The means values serum alkaline phosphate showed an increased trend in all the intoxicated birds. Shah *et al.* (1994) also reported elevation of serum alkaline phosphatase in rabbits after the oral adminstration of Ibuprofen. Similarly, Radino (1987) also reported increase in alkaline phosphatase levels after oral administration of Nemuslide in goats. The elevation of serum alkaline phosphatase level in the present study might be due to hepatotoxic effect of the NSAIDs.

There was no statistical difference in serum calcium between the intoxicated groups and control group. Similar observation has been reported (Prakesh and Nayak, 1999) after the oral adminstration of Diclofenac in birds. But, on the other hand, Dunn (2002) reported an increase in the calcium level in poultry after the administration of Ibuprofen which has been attributed to the increased activity of bone cells, however, no such effect was noticed in the present study.

No significant differences were observed regarding the serum BUN levels among intoxicated groups and control. However, an increase in the BUN level with diclofenac toxicity has been reported in rabbits (Sakr *et al.,* 1996), mice (Hickey and Ray, 2001), mongrel dogs (Ramesh and Narayana, 2002) and crossbred calves (Shridar, 2007). Similar results have been reported (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 contradictory results obtained could be attributed to the difference in the dosage level of drugs and duration of time used.

Relative weight of lymphoid organs

The mean values of relative weight of lymphoid organs viz. spleen, bursa of fabricius, thymus and caecal tonsils per kg body weight of birds is presented in Table 5. There was a significant decrease in the relative weight of spleen in intoxicated birds compared to control group expect in B1. The mean relative spleen weight of 2.5 ± 0.13 gm/kg body weight was recorded in control group. Among intoxicated groups highest atrophy of spleen was noticed in N3 and D3 with a value of 1.1 gm/kg body weight. Regarding the relative weight of bursa of fabricius, again a statistical difference was noticed among various treatment groups with weight decreasing in all the treatment groups. Highest value of 2.3 ± 0.17 gm/kg body weight was obtained in control group and lowest value (maximum atrophy) was recorded in B3 with a mean value of 1.20 ± 0.06 gm/kg body weight. The mean values for thymus also revealed statistical difference among treatment groups. Thymus weight increased in all the intoxicated groups except in N3 when compared to the control. Lowest value of 2.40 ± 0.24 gm/kg body weight was found in N3. The mean of caecal tonsils also showed statistical differences among various groups. Highest value of 3.50 ± 0.25 gm/kg body weight was observed in D1 and 2.3 ± 0.28 gm/kg body weight in B3. The mean value of control group was 2.50 ± 1.1 gm/kg body weight. In the present study, there was atrophy of spleen and bursa in intoxicated birds compared to the control group. The weights of thymus, bursa of fabricus and spleen can be used to assess the relative immune status in poultry (Rivas and Fabricants, 1988).

CONCLUSION

NSAIDs induced toxicity showed no effect on the haematological parameters of broiler chicks in the present study, however, there was increase in the serum alkaline phosphatase level in intoxicated birds compared to the

control group might be due to the hepatotoxic effect of NSAIDs. Further, atrophy of spleen and bursa of fabricius was observed in intoxicated birds which inturn depicts the immune status of the birds. There is a very scanty literature available regarding NSAIDs induced toxicity in broiler chicken, so further studies in this regard are required.

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