Original Article
Toxicology International, *May-Aug 2016 / Vol-23 / Issue-2, 134-139*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i2/146688

Safety Evaluation of Nimovet® in Poultry Birds

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Abstract

Nimesulide, a NSAID causes toxicity to poultry birds and indirectly to human through residue present in poultry meat. Birds of control and treated group (Gr-C, Gr-50, Gr-100 and Gr-200) received 10% ethanol, 50, 100 and 200 ppm Nimesulide (Nimovet®) in drinking water up to 42 days, respectively. Statistical analysis done by ANOVA and LSD by Turkey's test. No mortality was observed in treated groups. There was progressive and a significant reduction in feed and water intake in birds of Gr-200 at day 21, 28 and 42 as compared to control birds. The % body weight gain was significantly reduced in Gr-50 & Gr-100 at 21 day and Gr-200 from 14 to 42 day interval. Urea level was significantly increased in birds of Gr-50 &100 by 1.5 fold and Gr-200 by 2 fold as compared to the birds of Gr-C. The serum ALT, AST and ALP activity was significantly increased in the birds of Gr-200 as compared to birds of Gr-C. Histo-pathological alterations were observed in heart, liver, lungs and kidney leaving brain, intestine, thymus, spleen and bursa intact in the birds of Gr-200 at 42 day interval. Cumulative administration of Nimesulide (Nimovet®) in poultry birds is (i) safe up to 100 ppm for 6 week period and (ii) unsafe with organic toxicity at 200 ppm. In consideration of the present findings it could be predicted that Nimesulide (Nimovet®) at higher dose could cause toxicity to poultry birds itself and ultimate consumers including scavenger birds and animals.

Keywords: Nimesulide, Poultry birds, Toxicity, Safety evaluation

Received: 11-02-2016 **Revised:** 12-08-2016 **Accepted:** 19-08-2016

1. Introduction

Prostaglandins and COXs (Cyclo-oxygenases) participate in avian nociceptors and act peripherally and centrally similar to those of mammals[1]. It seems reasonable to expect that the NSAID would produce analgesia even in birds. Being a NSAID, it affects development of central nervous system, compromises the neural activity and corrects impairment of sciatic nerve morphology[2]. Nimesulide has been reported to be completely safe to use in birds than that of diclofenac[3] but subsequently this finding contradicted[4]. So, this controversy on safety of Nimesulide needs systematic evaluation of toxic effects on repeated oral administration in birds. Therefore, the present study designed to examine the safety evaluation of Nimesulide (Nimovet®) after cumulative oral administrations in synthetic strain of poultry birds.

2. Materials and methods

2.1 Experimental population

This experiment was approved by the Institute Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (Regd No. 433/CPCSEA/20-06-2001), Government of India prior its commencement. Forty apparently healthy poultry birds of synthetic strain (Vanaraja) purchased from Central Poultry Development Organization, Government of India, Bhubaneswar, at two weeks of age. They were allowed to acclimatize to the conditions of research poultry farm of this institute for two weeks. The birds were reared in four different cages with individual wing band, provided with free access to *ad- libitum* grower mash feed (Godrej) and piped drinking water without any multivitamin or anti-stress formulations during the experiment.

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2.2 Grouping and drug exposure

Experimental birds were randomly but equally divided into four groups, i.e, Gr-C (ID No-8690 to 8699), Gr-50 (ID No-8700 to 8709), Gr-100 (ID No-8710 to 8719) and Gr-200 (ID No-8720 to 8729). Nimesulide (Nimovet®) at the dose rate of 50, 100 and 200 ppm were prepared with drinking water afresh every day and provided to the birds in Gr-50 to 200, respectively. But, the birds in control group (Gr-C) received drinking water mixed with 10% ethanol as the vehicle control only.

2.3 Feed and water intake, body weight gain, Food Conversion Ratio (FCR) and mortality

Feed and water intake by individual bird of all groups were recorded daily from 0 to 42 day. Individual body weights of birds of all groups were recorded at 0, 7, 14, 21, 28, 35 and 42 day. A weekly body weight change of individual bird was recorded with respect to the weight at day 0. Cumulative FCR of each group was calculated from day 7 to 42 basing on the feed intake and body weight gain. Birds were observed daily for evaluation of clinical signs/ symptoms/ any behavioural alterations including depression/ alertness, response to disturbance and attraction to feed and palpation of joints to assess pain.

2.4 Haematology

Whole blood (2 mL) was collected from the jugular/wing vein in heparinized vials at 42nd day post-administration of Nimesulide (Nimovet®). The haematological parameters like Total Erythrocyte Count (TEC), haemoglobin concentration (Hb), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leukocyte Count (TLC) and Differential Count (DC) were studied.

2.5 Serum Biochemistry

Blood samples collected in sterile non-heparinized test tubes and allowed to clot and centrifuged at the rate of 3,000 revolutions per minutes (RPM) for 08 minutes for collection of serum and used for estimation of various biochemical parameters like serum Aspartate transaminase (AST), serum Alanine transaminase (ALT) and Alkaline phosphatase (ALP), Total protein, Albumin, Globulin, Glucose, Total cholesterol, Urea and Creatinine etc. by chemical kits.

2.6 Histopathology

The 10% neutral buffered formal saline solution fixed tissues were washed overnight in running tap water, dehydrated in ascending order of alcohol, and cleared in xylene. Paraffin blocks were prepared as per the routine procedure and sections were cut at 5-6 µ thickness followed by routine Haematoxylin and Eosin staining.

3. Statistical analysis

The data was subjected to statistical analysis according to Snedecor and Cochran (1980)[5] for Analysis of Variance (ANOVA) and least significant difference (LSD) by Turkey's test using Graphpad *Instat* 3 software.

4. Results

4.1 Feed and water intake, body weight gain, FCR and mortality:

The result of the effect of different doses of Nimovet® on percentage feed intake in poultry birds at different day's interval was presented in Table 1A. Table 1B depicted significant and progressive decline in water intake in Gr-200 birds at day 7 to 42. Mean percentage body weight gain was significantly decreased in birds of Gr-200 from day 14 to 42 and of Gr-50 &100 at day 21, as compared that of birds of Gr-C (Table 1C). The mean cumulative FCR was highly depressed in all treated as well as control groups at day 14. But it was attained to the control FCR from day 21 to 42 in all the groups (Fig 1).

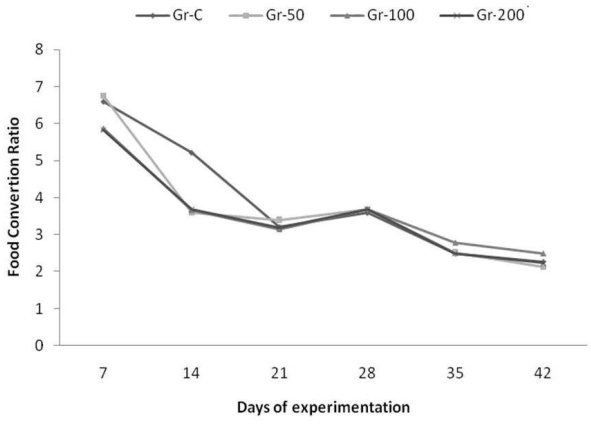


Figure 1. Cumulative feed conversion ratio (FCR).

Table 1 a. Effect of Nimovet® on percentages feed intake at different periods (day) in different treatment groups (Mean ± SEM), n=8

Periods (Day)	Gr-C	Gr-50	Gr-100	Gr-200
7	72.85 ± 4.42	75.77 ± 5.04	71.87 ± 3.35	68.85 ±2.40
14	77.96 ± 3.95	89.78 ± 2.63	89.66 ± 3.70	80.84 ±3.78
21	89.07 ± 3.28	89.51 ±2.09	86.22 ± 2.21	78.34 ± 2.66*
28	95.91 ± 0.90	97.46 ±0.78	94.82 ± 1.75	90.40± 1.93*
35	96.24 ± 1.88	91.35± 3.81	88.10 ± 5.31	91.55 ± 0.70*
42	99.38 ± 0.06	98.81±0.25	99.30 ± 0.12	92.63 ± 0.70*

Superscripts indicate significant variation (*p<0.05) between Nimovet® treated and control groups

Table 1 b. Effect of Nimovet® on percentages water intake at different periods (day) in different treatment groups (Mean ± SEM), n=8

Periods (Day)	Gr-C	Gr-50	Gr-100	Gr-200
7	57.43 ±2.70	49.79 ±2.86	52.29 ±2.57	34.43 ±1.71***
14	55.71 ±2.32	52.71 ±3.92	54.43 ±3.76	47.86 ±2.24**
21	67.57 ±3.76	63.07 ±5.92	58.14 ±4.98	60.64 ±1.45**
28	78.86 ±2.77	55.14 ±3.46	57.43 ±1.70	62.50 ±4.12**
35	78.93 ±5.02	78.71 ±2.14	76.29 ±4.78	54.36 ±6.24**
42	90.29 ±2.53	77.29 ±3.66	82.93 ±1.86	6.00***

Superscripts indicate significant variation (**p<0.01 and ***p<0.001) between Nimovet® treated and control groups.

Table 1 c. Effect of Nimovet® on percentages body weight gain at different periods (day) in different treatment groups (Mean ± SEM), n=8

Periods (Day)	Gr-C	Gr-50	Gr-100	Gr-200
7	14.54 ± 0.94	13.88 ±0.77	15.63 ±0.58	14.74 ± 0.62
14	39.44 ±3.45	37.87 ±3.31	32.43 ± 3.86	21.22 ± 1.21**
21	44.63 ± 1.02	33.11 ± 2.30***	29.10 ±1.52***	24.81 ±3.50***
28	47.89 ± 1.84	45.99 ±3.18	49.60 ± 1.63	34.51±6.51**
35	26.48± 1.79	25.44 ±3.01	22.85 ±0.61	21.70 ±1.39**
42	22.99± 4.11	19.81 ±2.16	16.97 ± 2.72**	11.67 ±2.54***

Superscripts indicate significant variation (**p<0.01 and ***p<0.001) between Nimovet® treated and control groups

4.2 Haematology

Haematological parameters of the experimental birds did not vary significantly between the groups (Table 2).

4.3 Serum Biochemistry

Significantly higher urea contents were estimated in birds of Gr-50 (p<0.01), 100 and 200 (p<0.001) than those of control ones (Table 3). The serum AST, ALT and ALP activities of Gr-200 birds were significantly (p<0.001) higher than those of Gr-C birds. Glucose, total protein, albumin, globulin, cholesterol and creatinine at day 42 did not vary significantly between the groups

4.4 Histopathology

The birds of Gr-200 following administration of Nimesulide for 42 days exhibited variable changes. The heart revealed myocardial congestion along with perivascular and intermycial edema (Fig 2). Liver was with diffuse sinusoidal congestion, but some cases exhibited hepatocytes disorganization, vascular degeneration, focal and perivascular infiltration of inflammatory cells (Fig 3). Interstitial congestion, edema with RBC infiltration in the para-bronchi and bronchial hyperplasia were manifested in lungs (Fig 4). Kidney exhibited interstitial congestion, mild hemorrhages, increased Bowman's space and vacuolar degeneration of tubular epithelium (Fig 5).

Table 2. Effect of Nimovet® on hematological parameters in different treatment groups (Mean ± SEM), n=8

Parameters (n=6)	Gr-C	Gr-50	Gr-100	Gr-200
TEC (× 10^6cu mm)	1.85±0.44	1.89±0.28	1.13±0.21	1.19±0.21
Hb (g dL^{-1})	9.58±0.34	10.40±0.65	8.83±0.37	8.77±0.25
PCV (%)	31.0±1.27	28.50±0.34	29.33±1.52	27.50±1.33
MCHC (pg dL^{-1})	36.50±1.98	37.33±2.20	38.0±2.31	39.50±0.56
TLC (× 10^3cu mm)	24.67±3.89	28.0±3.39	20.0±2.0	17.0±6.61
Neutrophil (×10^3cu mm)	55.33±2.45	54.0±2.52	51.33±1.58	49.83±0.83
Lymphocyte (×10^3cu mm)	6.83±0.87	7.17±0.65	9.0±0.63	8.50±0.34
Monocyte (×10^3cu mm)	1.33±0.21	1.89±0.17	1.66±0.21	1.83±0.17
Eosinophil (×10^3cu mm)	0.30±0.01	0.29±0.03	0.31±0.02	0.33±0.01
Basophil (×10^3cu mm)	1.14±0.07	1.15±0.05	1.16±0.04	1.18±0.02

Table 3. Effect of Nimovet® on different serum biochemical parameters in different treatment groups (Mean ± SEM), n=8

Parameters	Gr-C	Gr-50	Gr-100	Gr-200
Albumin (g dL^{-1})	2.12 ±0.18	2.31 ±0.11	2.28 ±0.16	2.15 ±0.08
Globulin (g dL−1)	2.81 ±0.22	3.19 ±0.08	2.54 ±0.31	2.72 ±0.24
Total protein (g dL^{-1})	4.92 ±0.23	5.50 ±0.05	4.82 ±0.21	4.90 ±0.18
Glucose (mg dL^{-1})	159.98±6.91	200.08±11.0	192.94±15.65	147.02±8.86
Cholesterol (mg dL^{-1})	89.07 ±4.63	73.12 ±4.86	75.61 ±8.36	89.42 ±2.39
Creatinine (mg dL^{-1})	1.00 ±0.09	1.10 ±0.03	1.03 ±0.03	0.89 ±0.03
Urea (mg dL^{-1})	23.19 ±4.11	37.82 ±2.29**	39.89 ±1.85***	41.81±1.28***
AST (IU L^{-1})	113.40±0.98	112.82±1.60	115.70±1.96	147.85±2.42***
ALT (IU L^{-1})	116.69±3.29	118.04±3.72	114.29±2.15	137.20±3.79***
ALP (IU L^{-1})	224.00±2.94	229.55±2.28	236.19±4.27	265.14±1.40***

Superscripts indicate significant variation (**p<0.01 and ***p<0.001) between Nimovet® treated and control groups

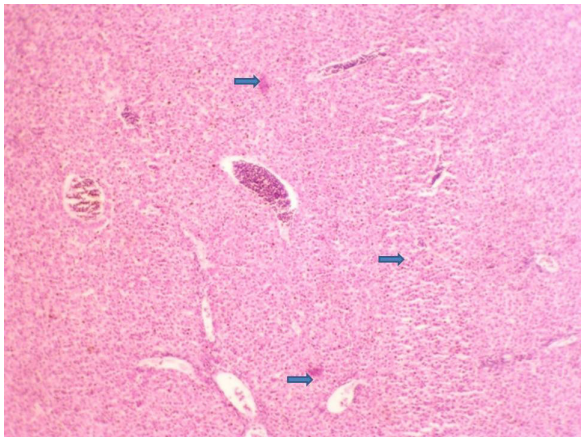


Figure 2. Diffuse sinusoidal congestion, hepatocytes disorganization, vascular degeneration, focal and perivascular infiltration of inflammatory cells in liver. H and E stain 200X.

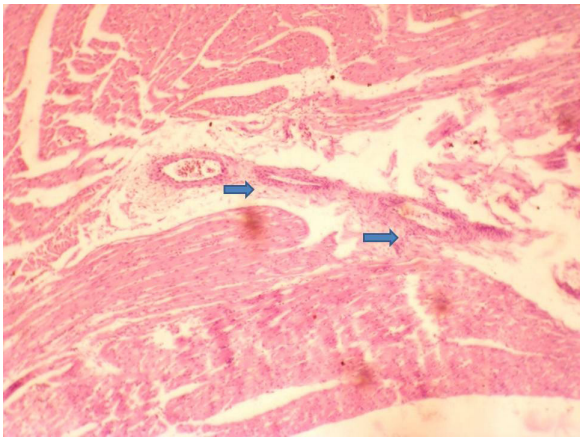


Figure 3. Myocardial congestion along with perivascular and intermycial edema changes in heart. H and E stain 200X.

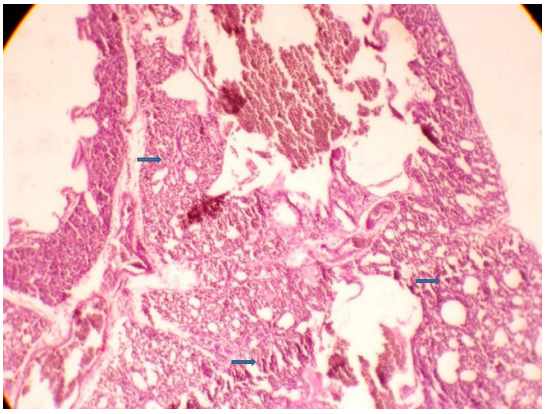


Figure 4. Interstitial congestion, edema with RBC infiltration in the para-bronchi and bronchial hyperplasia in lungs. H and E stain 200X.

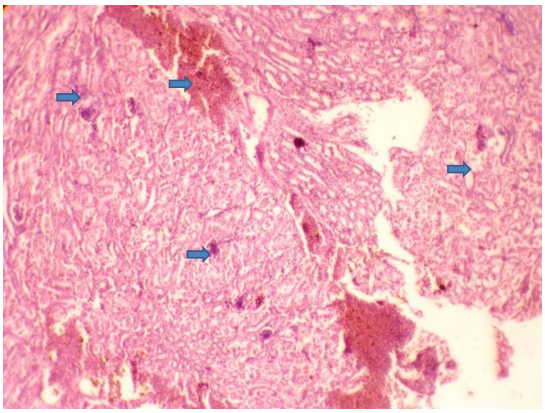


Figure 5. Interstitial congestion, mild hemorrhages, increased Bowman's space and vacuolar degeneration of tubular epithelium in kidney. H and E stain 200X.

5. Discussion

The major findings were that cumulative oral administration of Nimesulide (Nimovet®) in poultry birds cause significant (i) reduction in feed, water intake and body weight gain from day 21 to 42 (ii) increase in only serum urea in the birds of Gr-50 & 100 and increase in serum urea, AST, ALT and ALP in the birds of Gr-200 at day 42 and (iii) toxico-pathological alteration in the cells at day 42 in the order of severity liver> kidney> lungs> heart.

Observations of reduced water intake with oral Nimesulide (Nimovet®) in present study are in close resemblance with the findings of Flecknell et al. (1999)[6] in rats, though they used Caprofen, a NSAID which has similar mechanism of action as that of Nimesulide. Water requirement of meat birds depends on environment, diet composition, growth rate and efficiency of water reabsorption by the kidney. Even though, water and feed ratios stay relatively the same but increased feed intake and feed with high crude protein content enhances water intake in poultry birds[7]. Hence, reduced water intake by birds of Gr-200 could be attributed due to complementary effect arising from low feed consumption[8]. The birds of Gr-200 on exposure to 200 ppm dose exhibited reduced feed intake at peak period of growth on day 21, 28, 42 which may because Nimesulide causes depressed liver function in birds and interferes with gut motility[3]. Observations of reduced body weight gain with oral administration of Nimesulide in present study are in close resemblance with the findings of Allen (2000)[9] in chicks. As oral exposure of Nimesulide interferes with gut motility and causes depressed liver function in birds so as to alter the action of hepatic enzymes upon metabolism of food, may interfere in cumulative FCR values of different treated groups which reflect directly on reduction in percentage body weight gain.

The oral dose up to 200 ppm Nimovet® for 42 days did not induce any severe toxic signs/ symptoms and mortality. This result holds well with Swetha et al. (2005)[10] as no mortality was evinced in the broiler chickens administered intramuscularly with Nimesulide at the dose rate of 2 mg kg^{-1} body weight. Reddy et al. (2006)[3] also reported no mortality in a synthetic poultry bird (Vanaraja) for Nimesulide treated (2 and 5 mg kg^{-1} body weight) group. Hence maximum tolerated dose (MTD) of Nimovet® would be more than 200 ppm in poultry birds. It implies that, the haematological parameters are not affected by Nimesulide up to 200 ppm dose in birds as evident from the results of the haematological parameters of the experimental birds in this study did not vary significantly between the groups. The serum biochemical constituents of experimental birds for glucose, total protein, albumin, globulin, cholesterol and creatinine at day 42 did not vary significantly between the groups which corroborates with Swetha et al. (2005)[10] and Reddy et al. (2006)[3]. There was no report regarding the values of serum glucose and serum protein fractions.

We observed higher urea contents in birds of Gr-50 ($p<0.01$), 100 and 200 ($p<0.001$) than those of control ones (Table 3). Significantly higher urea contents were estimated in birds of all tested groups than those of control ones in present study may be due to significant decrease in water intake during treatment, so dehydration may take place to combat the homeostasis of the body. Unlike uric

acid, blood urea concentrations are more significantly affected by the bird's hydration status. Separate studies involving the domestic fowl and pigeons demonstrated decreased urea elimination and/ or increased blood urea levels (6.5-15.3 fold increase in pigeons) in dehydrated birds[11]. During normal hydration, filtered urea is 100% excreted but is 99% reabsorbed in the tubules during dehydration[12].

Elevated levels of AST, ALT and ALP in serum of birds under highest dose rate (200 ppm) than in control birds of no drug treatment may attribute to the alteration in architectural integrity of hepatocytes and muscle fibres due to induction of oxidative stress or apoptosis or damage of mitochondria by major mechanisms of uncoupling of oxidative phosphorylation[13]. A dose of 200 ppm Nimesulide induces leakage of liver specific enzymes into serum stating alteration of liver function. But the dose of 150 ppm and above can alter kidney function to intervene in nitrogen metabolism and urea retention[14].

Histopathology revealed that, in birds of Gr-200, after administration of Nimesulide for 42 days liver exhibited diffuse sinusoidal congestion, hepatocyte disorganisation, vascular degeneration, focal and perivascular infiltration of inflammatory cells which may be due to induction of oxidative stress and formation of reactive metabolites which bind to proteins[15]. In our result, kidney exhibits interstitial congestion, mild haemorrhages, increased Bowman's space and vacuolar degeneration of tubular epithelium which may be due to the mitochondrial toxicity leading to activation of cell death-signalling pathways. Thus, Nimuslide (Nimovet®) at 200 ppm can induce cellular changes in heart, liver, lungs and kidney.

From observations of this study it could be stated that cumulative accumulation of Nimesulide (Nimovet®) beyond certain concentration would cause toxicity to poultry birds it' self and ultimate consumers including scavenger birds and animals. So deserves a warning for its safety on disposal to environment as well as administration to birds as therapeutic agent.

6. Conflict of Interest

None Declared

7. References

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