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Short Communication A SCITECHNOL JOURNAL

Differences in NSAID Toxicity in Four Avian Species

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Abstract

The non-steroid anti-inflammatory drugs (NSAIDs) are clinically applied in birds however their impact in birds is unknown. The aim of this study was to investigate the pharmaco-toxicological effect of diclofenac and acetyl-salicylic acid in four avian species, including broiler chickens, domestic pigeons, budgerigars and common quails. Our goal was to determine the effect of water deprivation to the investigated active agents' toxicology in all four species. Diclofenac (5 and 50 mg/kg bw.) and acetyl-salicylic acid (50 mg/kg bw.) were administered orally to four birds per group of each species for 3 days. Twelve birds were used of each species; all of them were partially deprived of water (20 ml/kg bw). Another twelve broiler chickens had free access to water to compare the impact of partial water deprivation to NSAID exposure. Both doses of diclofenac caused harmful effects in the examined species. Six of eight broiler chickens died after the second exposure, the cause of death was serious visceral gout. The high dose diclofenac caused death in all four species; pigeon was the least sensitive to diclofenac. The water-deprivation significantly accelerated the mortality of chickens. The acetyl-salicylic acid was clinically well-tolerated in all examined species.

Keywords: Birds; Dehydration; Kidney; NSAID toxicity

Short Communication

The non-steroid anti-inflammatory drugs (NSAID) are the most frequently used analgesic agents in small animal practice. The acetyl-salicylic acid and the diclofenac were the objects of our investigations. These agents are approved veterinary drugs, even in livestock production. The acetyl-salicylic acid is the only NSAID which is licensed for application in birds even in poultry farming. Furthermore they are characteristic active ingredients commonly used in human medicine. These human licensed products are typical over the counter (OTC) drugs; uninitiated use in bird species is highly possible. Numerous NSAIDs are recommended to use in avian species [1], although the effect of NSAIDs in bird species is a blank field in the scientific literature [2]. The application and dosages of several NSAID formulations continues to be clinically applied in birds [3]. The decline of the Asian vultures [4,5] called the attention for the danger of the use of NSAIDs in birds.

The aim of our study is to investigate the toxic effect the short-term use of 5 mg/kg bw, 50 mg/kg bw, Diclofenac and 50 mg/kg bw acetyl-salicylic acid in four avian species, furthermore to examine the effect of the water deprivation on the NSAID exposure. Partial water deprivation was applied because reduced water intake can occur when the bird feels pain or suffer from a disease and due to dehydrated condition glomerular filtration rate will be decreased [6]. When the glomerular filtration rate is reduced the excretion is disturbed so the drug elimination will be inhibited and this leads to more harmful consequences.

The animal trials were conducted according to approved laboratory animal experimentation ethics regarding to the national and European law. The study was authorized by the Local Institutional Animal Care Committee (no. 40/2014).

Four bird species were used in our study; broiler chickens (*Gallus gallus*), domestic pigeons (*Columba livia domestica*), budgerigars (*Melopsittacus undulatus*) and common quails (*Coturnix coturnix*). A total of 72 clinically healthy birds of mixed gender were used (Table 1), 12 treated birds plus 3 controls of each species. The animals were housed in stainless steel cages four birds per cages, acclimated at 20 ± 2ºC on a 12 h light 12 h dark schedule. Feed was provided *ad libitum* and all birds were partially deprived of water; 20 ml/kg bw per day. The birds of each species were randomly divided into 4 experimental groups. The control groups were treated with deionized water; the other three groups were treated orally with 5 mg/kg or 50 mg/kg diclofenac or with 50 mg/kg acetyl-salicylic acid for three consecutive days. Twice as much broiler chickens were treated, half of them were partially deprived of water and another 12 birds had drinking water *ad libitum*. Six hours after the first diclofenac application and 6 h after the second application blood samples were collected; calcium, phosphorus, carbamide, uric acid and total protein were measured. On the fourth day i.e. a day after the last NSAID application the birds were euthanized. Kidney and liver samples were collected and fixed in 8% neutral buffered formalin for 24 h and embedded in paraffin wax. Sections (3–4 µm) were cut, stained with haematoxylin and eosin, and examined by light microscopy. Statistical analyses were performed by Statistica 12 software (Statsoft, Tulsa, USA). Differences between means were evaluated by one-way analysis of variance (ANOVA) followed by a post hoc comparison using Fisher's least significant difference (LSD) test. Survival curves were calculated using the Kaplan-Meier method, the curves were compared using the Cox's F test. Statistical significance was set at $p<0.05$.

	Body weight (g) (mean ± SD)	Gender ratio
Broiler chickens (water *ad libitum*)	1105 ± 113	6 male, 6 female
Broiler chickens	1262 ± 258	8 male, 7 female
Pigeons	394 ± 28	8 male, 7 female
Quails	82 ± 14	7 male, 8 female
Budgerigars	39 ± 3	9 male, 6 female

Table 1: Body weight and gender ratio of the used birds by species All birds were partially deprived of water (20 ml/kg bw.) during the three day long treatment period except where otherwise indicated

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The acetyl-salicylic acid treated birds remained clinically healthy, only microscopic alterations were detectable. In the kidneys of 2/4 quails, 2/4 budgerigars, 4/4 chickens and 4/4 pigeons as well as the 4/4 chickens which had free access to water, the junction structures between renal tubular epithelial cells vanished so the tubular cells have dissociated, the structure of the tubule was unrecognizable. The nuclei were necrotic, the nuclear envelope showed hyperchromasia, it stained darker and DNA was adhered to it and nuclear shrinkage and nuclear disintegration were observed. In the renal tissue leukocyte infiltration was detected. In the liver samples alterations were not observed.

The diclofenac treated birds presented with symptoms of lethargy, weakness and ruffled feathers, 18 birds died during or after the treatment period. The post-mortem findings of these birds were serious uricosis and visceral gout in each species. Diclofenac caused mortality in each species as shown in Figure 1. The low dose (5 mg/kg) diclofenac treatment caused low mortality rate compare to the high dose (50 mg/kg) diclofenac treatment (F=3.265, p=0.010). Chicken was most sensitive to diclofenac and pigeon was the least sensitive considering the four examined species.

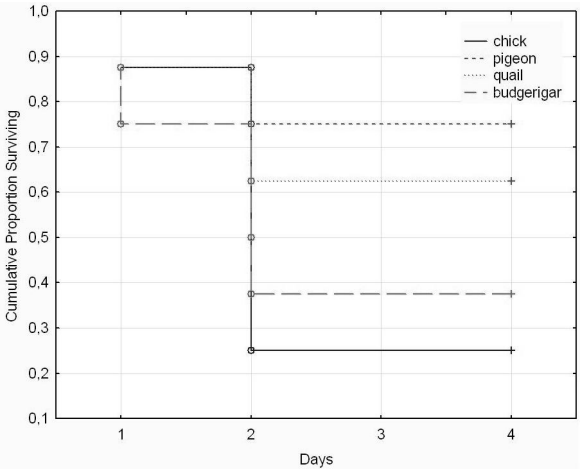

Figure 1: Impact of diclofenac treatment in four avian species. Birds were received either 5 mg/kg bw (n=4) or 50 mg/kg bw (n=4) diclofenac. Curves were calculated by Kaplan-Meier method and Cox's F test was used for comparisons between treatments. Chicken x pigeon (F=4.670, p=0.016), pigeon x quail (F=1.708, p=0.265), pigeon x budgerigar (F=3.587, p=0.046), chicken x budgerigar (F=1.230, p=0.362), budgerigar x quail (F=2.076, p=0.147), chicken x quail (F=2.641, p=0.072)

Regarding the results of the applied diclofenac along with partial water deprivation; fifteen diclofenac treated birds died during the treatment period as follows in detail.

Following 50 mg/kg diclofenac administration 4/4 broilers, 3/4 budgerigars, 3/4 quails and 1/4 pigeon died during the treatment period. In the kidneys of 4/4 chickens (Figure 2), 4/4 budgerigars (Figure 2), 3/4 quails and 1/4 pigeon serious tubulonephrosis and marked tubular epithelial necrosis were observed. The livers of the 50 mg/kg diclofenac treated birds (4/4 chickens, 4/4 budgerigars, 3/4 quails and 1/4 pigeon) showed granulocyte infiltration, cytoplasmic vacuolization and some necrotic hepatocyte cells appeared.

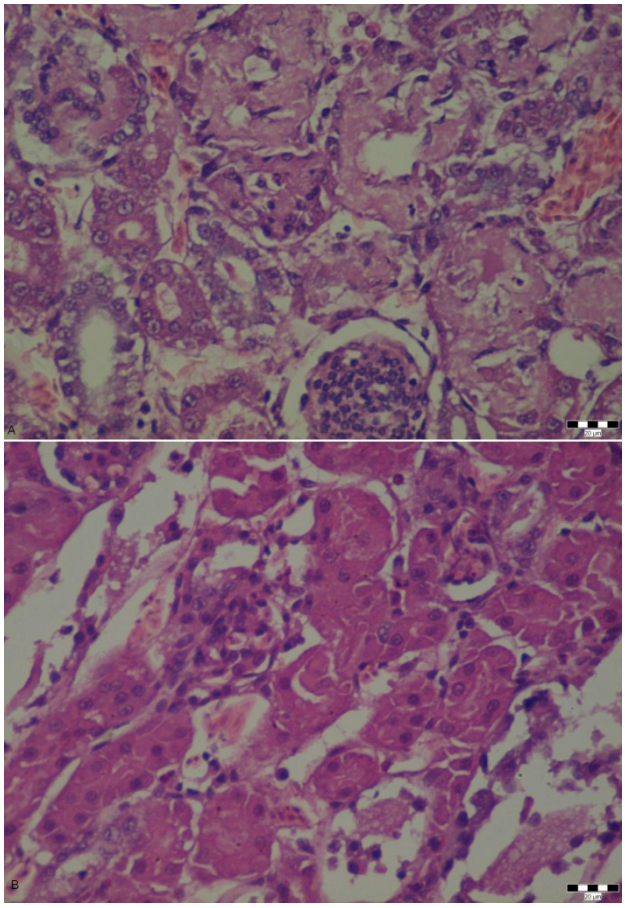

Figure 2: Representative photomicrographs of renal tissue from chicken (a) and budgerigar (b) treated with 50 mg/kg bw Diclofenac show tubular degeneration, severe tubular necrosis, dilated tubules with flattened epithelial lining, marked inflammatory alterations and interstitial fibrosis. Hematoxylin and eosin stain; magnification, 200x.

Following 5 mg/kg diclofenac administration 2/4 broiler chickens and 2/4 budgerigars died during the treatment period. In the kidneys of 4/4 chickens and 4/4 budgerigars tubulonephrosis and acute tubular epithelial necrosis were detected. In the liver samples of 4/4 chickens and 4/4 budgerigars granulocyte infiltration and cytoplasmic vacuolization were observed. The tissue samples of the 5 mg/kg diclofenac treated quails and pigeons remained intact.

Regarding the results of the applied diclofenac with free access to water three diclofenac treated birds died during the treatment period. Three of the four 50 mg/kg diclofenac treated chickens, which were provided with water *ad libitum*, died during the treatment period. The cause of death and the histopathological findings were the same as in the water-deprived chickens. Following 50 mg/kg diclofenac administration 4/4 chickens and 5 mg/kg diclofenac administration 2/4 chickens were observed to have tubulonephrosis and tubular epithelial necrosis in the kidneys. Nonetheless the death occurred approximately one day later in the chickens which had free access to water than the water-deprived chickens (F=3.371, p=0.03). Regarding the results of the blood analysis, the serum phosphorus level increased

($p<0.05$) 6 hours after the first application and the serum uric acid level increased ($p<0.01$) 6 hours after the second application in both the low and the high dose diclofenac treated groups.

Gender differences in the effect of diclofenac and acetyl-salicylic treatment were not detectable; deaths, microscopically and microscopically alterations occurred in both gender.

In conclusion short-term acetyl-salicylic acid treatment - less than or equal to 50 mg/kg bw dose - is safe in every examined bird species. Nevertheless the five day long application of this agent can cause lesions in the intestinal mucosa as well as severe congestion and hemorrhages in the small intestine [7], furthermore the long-term application of acetyl-salicylic acid can lead irreversible degenerative changes in bone tissues [8].

Diclofenac caused renal impairment in the four examined species. This finding is consistent with the results of Hussain et al. [9] although in their study pigeons found to be more sensitive to diclofenac in contrast to our findings, however according to them on day 21 the survivor individuals fully recovered from the 7 day long 10 mg/kg bw Diclofenac application.

Glomerular filtration is not constant in birds, and is considerably lower in dehydrated animals [10], moreover there is a significant difference between bird species regarding to NSAID sensitivity, for this reason their impact should be examined in each bird species [11].

Overall the use of diclofenac even in 5 mg/kg bw represents a high risk in budgerigars and broiler chickens, especially in dehydrated condition. Nonetheless according to our findings the three day long administration of the lower dose diclofenac (5 mg/kg) was tolerable in quails and pigeons. However rehydration should be applied during the treatment period.

Avian species are significant in food production and some of them are popular pet animals that necessitate the effective and safe pain control in these species. Besides diclofenac other NSAID agents cause harmful effects in different bird species [12], according to these findings prior to the actual treatment, test treatment should be applied in birds to avoid the unpredictably adverse effects or fatal outcome. More studies are needed for detection of interspecies differences in safety of various NSAIDs in birds.

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