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Effects on hematology and blood biochemistry profile of intramuscular meloxicam injection in Brahminy kite and Barn owl

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ABSTRACT

Background: Meloxicam is used widely for exotic animal analgesia, but its toxicity in common raptor species in Thailand is unclear.
Objectives: This study evaluated the single-dose effect of intramuscular meloxicam in common raptor species in Thailand for short-term and long-term periods.
Methods: Twenty-two raptors were administered a single 1 mg/kg dose of meloxicam individually via intramuscular injection. The following were evaluated: clinical appearance, body weight, body condition score, body temperature, fecal appearance, complete blood cell count, and biochemistry panel before (day 0) and after the injection (1, 7, and 30 days). The collected samples were categorized into three groups: Brahminy kite (*Haliastur indus*) (n = 10), adult Barn owl (*Tyto javanica*) (n = 4), and juvenile Barn owl (n = 8).
Results: None of the raptors in the study groups showed any abnormalities. The hematological profiles were significantly different in the short-term period (day 1 and day 7). The creatinine, aspartate aminotransferase, and creatinine kinase increased in several groups. On the other hand, the packed cell volume decreased in the Brahminy kite and juvenile Barn owl groups. According to the findings, an intramuscular injection of 1 mg/kg meloxicam affected the blood biochemistry panel of the muscle, but the affected raptors recovered within one week.
Conclusions: An intramuscular injection of meloxicam at a single 1 mg/kg dose in Brahminy kites and Barn owls was not associated with the morbidity, hepatotoxicity, gastrointestinal toxicity, and nephrotoxicity in the short- and long-term periods.

Keywords: NSAIDS; raptors; blood cell count; blood chemical analysis; birds

INTRODUCTION

Analgesia has become a standard and appropriate practice in avian medicine [1]. Various analgesic drugs are used in birds, consisting of opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), local anesthetics, and providing multimodal protocols with other drugs acting at different points in the nociceptive system [2]. NSAIDs have often been used to

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Conflict of Interest
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relieve acute and chronic pain in the musculoskeletal and visceral organs [3]. The adverse effects of NSAIDs are dose-dependent. The most common adverse effects of NSAIDs in mammals are related to the gastrointestinal, renal, and coagulation systems. The negative impacts on renal tissue and function appear to be the most prevalent side effect of using NSAIDs in avian species. Those consequences can be severe and lead rapidly to renal failure and visceral gout [1,4]. The kidney uses both cyclooxygenase (COX)-1 and COX-2 for prostaglandin synthesis; renal injury occurs when renal prostaglandin synthesis is inhibited [3]. COX-2-selective NSAIDs are used wildly in avian medicine based on the hypothesis that the inhibition of COX-1 has renal adverse effects. On the other hand, COX-2 metabolites have been implicated in maintaining renal blood flow, mediating renin release, and regulating sodium excretion. Therefore, those selective COX-2 inhibition NSAIDs that interfere with COX-2 activity can adversely affect the renal blood flow and glomerular filtration rate [4].

NSAIDs also play a significant role in renal toxicity in raptors, as in other bird species, particularly flunixin meglumine and carprofen. On the other hand, there are no reports of renal toxicity with meloxicam in 39 individuals from six species of Gyps vultures and a minimum of 700 birds from 54 other raptors and scavenging species at 0.1–0.75 mg/kg dosage [5]. Meloxicam, a COX-2-preferential NSAID, which inhibits COX-2 rather than COX-1 [6], is used wildly in exotic and avian medicine because of its fewer adverse effects than other drugs. According to the study on American kestrels, the high dosage of oral meloxicam administration could lead to hepatic lipidosis and gastric ulcer without the consequence of nephrotoxicity after administrating oral meloxicam at 20 mg/kg dosage for seven days. Nevertheless, the long-term effects of using meloxicam were not evident in the study [7]. Consequently, this study evaluated the short- and long-term effects of the meloxicam injection in Brahminy kites and Barn owls, the common raptor species in Thailand, at a 1 mg/kg dosage using the physical examination, hematology, and blood biochemistry profile.

MATERIALS AND METHODS

Population

Twenty-two raptors, unknown sex, in two families, were used in this study: the *Accipitridae* family, Brahminy kite (*Haliastur indus*) (n = 10); the Strigidae family, Barn owl (*Tyto javanica*) (n = 12). All raptors had been admitted to Kasetsart University Raptor Rehabilitation Unit (Thailand). All Brahminy kites were adults. The Barn owls were divided into two groups. The adult Barn owl group (n = 4) were disabled and non-releasable due to a wing injury and were kept as education birds. The juvenile Barn owl group (n = 8) were orphaned birds.

Before the protocol started, all birds received a complete physical examination by a veterinarian and initial blood testing for packed cell volume (PCV) and plasma protein. All birds showed normal clinical appearance and behavior. The PCV and plasma protein were in the normal reference range [8,9]. The results suggested that they were healthy.

Outdoor rehabilitation enclosures were used to house the birds. During the study period, all the birds were fed frozen-thaw day-old chicks *ad libitum* every other day. Before blood collection, they were fasted for 24 h but were provided water *ad libitum*. The Institutional Animal Care and Use Committee, Kasetsart University, Thailand (approval No. ACKU64-VET-052) approved the animal care and procedures.

Study design

Each bird was injected with meloxicam (1 mg/kg IM once, Metacam; Boehringer Ingelheim Vetmedica GmbH, Germany) at the pectoral muscle. The selected dosage was based on previous studies that used meloxicam in raptors [7,10]. Subsequently, the physical examination and blood collection were made before the injection (day 0) and collected repeatedly on 1, 7, and 30 days after administration.

Three clinical parameters were collected during a physical examination, including body weight, body condition score, and body temperature. The investigation of the mental status, such as alertness to the presence of a human caretaker, preening, defecating, flying in a large flight enclosure, appetite, and fecal appearance, was performed once daily for 30 days throughout the study period. Blood collection was performed in the morning after 24 hours of fasting. Blood (1.2–1.5 mL) was collected via the right jugular vein, then separated into three samples: 0.5 mL, 0.3 mL, and the remaining volume. A 0.5 mL blood sample was transferred to K3 EDTA microtube to evaluate PCV, plasma protein, and complete blood cells count. Another 0.3 mL blood sample was transferred to a lithium heparin tube for ammonia detection. The remaining blood sample was placed in a serum clot activator tube for the biochemical analysis of creatinine, total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine kinase (CK), and uric acid. PCV was evaluated using the microhematocrit method. In contrast, microcentrifuge was set to 12,000 RPM for 5 min and read on PCV standard chart, and a Goldberg refractometer was used for the plasma protein measurement. Furthermore, the glass slide smears were stained with Wright's stain for the white blood cell (WBC) differential count. A Natt and Herrick's solution was used to count the red blood cell (RBC) and WBC directly. Finally, the blood biochemistry values were evaluated using a spectrophotometry technique and dry-slide technique in the laboratory unit of Kasetsart University Veterinary Teaching Hospital, Thailand.

Statistical analysis

The data were divided into three groups: Brahminy kite (*Haliastur indus*) (n = 10), Adult Barn owl (*Tyto javanica*) (n = 4), and juvenile Barn owl (*Tyto javanica*) (n = 8) with four time periods including before (day 0) and after the administration of meloxicam (day 1, day 7, and day 30). All parameters, including body weight, body temperature, PCV, plasma protein, hemoglobin, RBC, WBC, thrombocyte, creatinine, total protein, albumin, AST, ALP, CK, uric acid, and ammonia, were analyzed using repeated-measure analysis of variance. A least significant difference test was used between before (day 0) and after injection (day 1, day 7, and day 30) to define the difference in the parameter values. All statistical analyses were performed on IBM SPSS Statistics 25 program (IBM Corp., USA) with a $p < 0.05$ statistical significance level.

RESULTS

None of the birds showed any abnormal clinical signs throughout the study period. All raptors studied showed a normal mental status after the study was finished. The bodyweight, body condition score, and body temperature were similar in the meloxicam pre- and post-injection groups ($p < 0.05$; **Table 1**). All birds had a normal fecal appearance in every period of this study. The birds had a normal appetite except for two Brahminy kites that showed a decreased appetite for three days after the meloxicam administration.

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Table 1. Body weight and body temperature of Brahminy kite (n = 10), adult Barn owl (n = 4), and juvenile Barn owl (n = 8) before (day 0) and after (day 1, day 7, and day 30) intramuscular injection of 1 mg/kg meloxicam

Analyte	Day 0	Day 1	Day 7	Day 30
Body weight (g)				
Brahminy kite	501.5 ± 73.9	500.5 ± 72.5	496 ± 61.9	507 ± 73.3
Barn owl (A)	412.5 ± 53.5	405.5 ± 52.6	423.7 ± 55.9	427.5 ± 51.7
Barn owl (J)	407.5 ± 32.9	411.9 ± 39.1	401.3 ± 36.8	424.4 ± 27.7
Body temperature (°C)				
Brahminy kite	41.6 ± 0.5	41.6 ± 0.6	41.3 ± 0.5	41.9 ± 0.8
Barn owl (A)	40.3 ± 1.2	40.5 ± 0.9	40.4 ± 0.8	40.5 ± 0.9
Barn owl (J)	40.1 ± 0.7	40.5 ± 0.6	40.6 ± 0.9	40.6 ± 0.8

Values are presented as mean ± SD.
*Values on Day 1, 7 and 30 are not significantly different from those on Day 0.

Regarding the hematology profile, the mean PCV in the Brahminy kite and juvenile Barn owl groups decreased significantly on day one after treatment and recovered one week later. The mean plasma protein increased on day 7 and day 30 in the Brahminy kite and juvenile Barn owl groups, respectively. The mean thrombocyte value in the juvenile Barn owl group decreased on day 1 and day 30, as shown in **Table 2**. The hemoglobin, RBC, WBC, heterophil, lymphocyte, eosinophil, basophil, and monocyte levels were similar, as shown in **Tables 2** and **3**.

The blood biochemistry profile showed no differences in total protein, albumin, ALP, uric acid, and ammonia values, as shown in **Table 4**. The creatinine values increased in the Brahminy kite group on day 1. The AST level increased significantly in the Brahminy kite and juvenile Barn owl groups on day 1. In the Brahminy kite group, the AST was still high on day seven and decreased slightly to the basal level on day 30. The CK values increased in the juvenile Barn owl group only on day 1.

Table 2. Complete blood count of Brahminy kite (n = 10), adult Barn owl (n = 4), and juvenile Barn owl (n = 8) before (day 0) and after (day 1, day 7, and day 30) intramuscular injection of 1 mg/kg meloxicam

Analyte	Day 0	Day 1	Day 7	Day 30
Packed cell volume (%)				
Brahminy kite	44 ± 3	42 ± 2*	45 ± 4	43 ± 3
Barn owl (A)	42 ± 6	39 ± 1	40 ± 3	43 ± 4
Barn owl (J)	46 ± 3	42 ± 4*	45 ± 3	49 ± 4
Plasma protein (g/dL)				
Brahminy kite	5.1 ± 0.6	5.1 ± 0.6	5.3 ± 0.6*	4.8 ± 0.5
Barn owl (A)	4.4 ± 0.3	3.9 ± 0.3	5.3 ± 0.6	4.8 ± 0.5
Barn owl (J)	4.3 ± 0.5	4.7 ± 0.8	4.5 ± 0.3	5.1 ± 0.7*
Hemoglobin (g/dL)				
Brahminy kite	14.3 ± 1.6	14.7 ± 3.4	14.2 ± 1.6	14.2 ± 1.2
Barn owl (A)	11.8 ± 1.2	11.3 ± 0.8	11.3 ± 1.1	11.8 ± 0.8
Barn owl (J)	13 ± 0.4	12.4 ± 0.5	12.8 ± 0.6	12.7 ± 0.8
Red blood cell ($\times 10^6/\mu L$)				
Brahminy kite	2.7 ± 1.4	2.5 ± 1.1	4.2 ± 2.2	3.6 ± 1.4
Barn owl (A)	3.9 ± 0.5	3.1 ± 1.1	3.3 ± 0.5	3 ± 0.9
Barn owl (J)	3.9 ± 0.5	3.8 ± 0.4	3.9 ± 0.6	3.4 ± 0.9
WBC ($\times 10^3/\mu L$)				
Brahminy kite	10.3 ± 6.7	12.7 ± 7.1	14.2 ± 5.3	15.2 ± 6.4
Barn owl (A)	13.3 ± 4.5	9.5 ± 2.9	19.3 ± 7.5	22.5 ± 14.3
Barn owl (J)	16.3 ± 5.5	19.1 ± 4.6	16.3 ± 6.9	15.4 ± 2.2
Thrombocyte (%WBC)				
Brahminy kite	223 ± 76	215 ± 88	235 ± 49	219 ± 68
Barn owl (A)	269 ± 128	201 ± 89	210 ± 107	218 ± 84
Barn owl (J)	302 ± 50	258 ± 55*	282 ± 50	214 ± 38*

Values are presented as mean ± SD.
WBC, white blood cell.
*Values differ significantly from day 0 ($p < 0.05$).

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Table 3. White blood cell count of Brahminy kite (n = 10), adult Barn owl (n = 4), and juvenile Barn owl (n = 8) prior to (day 0) and after (day 1, day 7, and day 30) intramuscular injection of 1 mg/kg meloxicam

Analyte	Day 0	Day 1	Day 7	Day 30
Heterophil (×10³/μL)				
Brahminy kite	7.5 ± 5.1	9 ± 5.4	9.9 ± 4.2	10.4 ± 5.3
Barn owl (A)	8.8 ± 3.3	6.1 ± 2	12.6 ± 6.1	12.3 ± 6.6
Barn owl (J)	11.1 ± 4.1	12.3 ± 5	11.8 ± 5.5	10.3 ± 2.1
Lymphocyte (×10³/μL)				
Brahminy kite	1.6 ± 1.2	1.8 ± 1.1	1.8 ± 0.9	1.5 ± 0.9
Barn owl (A)	3.8 ± 2.3	3 ± 1.3	5.1 ± 2	7.8 ± 5.5
Barn owl (J)	3.7 ± 1.6	3.4 ± 1.2	3.4 ± 1	2.8 ± 0.8
Eosinophil (×10³/μL)				
Brahminy kite	0.9 ± 0.6	1.2 ± 1.1	1.8 ± 1.9	2.5 ± 2.2
Barn owl (A)	1 ± 0.3	0.8 ± 0.2	1.1 ± 0.7	1 ± 0.5
Barn owl (J)	1.1 ± 1	0.9 ± 0.5	0.9 ± 0.6	1.8 ± 0.1
Basophil (×10³/μL)				
Brahminy kite	0.25 ± 0.31	0.24 ± 0.21	0.51 ± 0.28	0.64 ± 0.54
Barn owl (A)	0.74 ± 0.15	0.15 ± 0.15	0.61 ± 0.4	0.75 ± 0.61
Barn owl (J)	0.29 ± 0.22	0.38 ± 0.17	0.27 ± 0.27	0.48 ± 0.26
Monocyte				
Brahminy kite	0.14 ± 0.17	0.23 ± 0.23	0.07 ± 0.12	0.1 ± 0.15
Barn owl (A)	0.03 ± 0.07	0.23 ± 0.24	0	0
Barn owl (J)	0.05 ± 0.14	0.06 ± 0.1	0.02 ± 0.05	0.03 ± 0.09

Values are presented as mean ± SD.
*Values on Day 1, 7 and 30 are not significantly different from those on Day 0.

DISCUSSION

None of the birds showed any adverse signs in the short- and long-term periods after administration and no clinical signs of gastrointestinal toxicity, such as decreasing appetite, diarrhea, and melena in any group of birds. On the other hand, two individuals of Brahminy kite that showed a decrease in appetite for three days after the meloxicam administration may be attributed to stress from transferring birds between a large flight enclosure and a smaller observation cage during the study period. On day 1, the mean PCV decreased in all groups and differed significantly in the Brahminy kite and juvenile Barn owl groups. This suggests blood loss and hemolytic or depression anemia [7]. The decrease in the mean PCV after administrating meloxicam was also found in previous studies on meloxicam toxicity in the American kestrel [7]. Furthermore, a mild increase in the mean PCV in the adult Barn owl group on day 30 may result from mild dehydration, as evident with an increase in plasma protein, but it was still in the normal reference range [11,12]. On the other hand, employing birds in an outdoor enclosure during the study period may cause mild dehydration on day 30 in adult Barn owls.

The thrombocyte count decreased significantly on day 1 and day 30 without any abnormal clinical signs and was still within the normal reference range in the juvenile Barn owl group [13]. NSAIDs can impair the platelet function by inhibiting platelet COX and blocking the formation of thromboxane A2 [14]. NSAIDs also induce thrombocytopenia in animals that receive aspirin [15]. Moreover, there are few documented case reports of thrombocytopenia in humans that may be induced by meloxicam. These reports also documented abnormal clinical appearances, including melena, bruising, or bleeding [16,17]. The decrease in thrombocyte count on day one can cause a subcutaneous hematoma at the blood collection site, *i.e.*, jugular vein, during the study period. On the other hand, the cause of the decrease in thrombocyte count on day 30 requires further study.

Table 4. Blood biochemistry of Brahminy kite (n = 10), adult Barn owl (n = 4), and juvenile Barn owl (n = 8) before (day 0) and after (day 1, day 7, and day 30) intramuscular injection of 1 mg/kg meloxicam

Analyte	Day 0	Day 1	Day 7	Day 30
Creatinine (mg%)				
Brahminy kite	0.4 ± 0.1	0.6 ± 0.1*	0.5 ± 0.1	0.4 ± 0.2
Barn owl (A)	0.6 ± 0.04	0.6 ± 0.08	0.5 ± 0.2	0.5 ± 0.1
Barn owl (J)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Total protein (gm%)				
Brahminy kite	4 ± 0.7	4.7 ± 2.1	4.3 ± 0.5	3.7 ± 0.6
Barn owl (A)	3.5 ± 0.1	3.1 ± 0.3	3.3 ± 0.6	3.9 ± 0.4
Barn owl (J)	3.3 ± 0.3	0.4 ± 0.4	3.1 ± 0.7	3.4 ± 0.9
Albumin (gm%)				
Brahminy kite	1.6 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.2
Barn owl (A)	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.2	1.7 ± 0.2
Barn owl (J)	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
Aspartate aminotransferase (U/L)				
Brahminy kite	361.2 ± 158.4	603.9 ± 238.7*	503.9 ± 171.8*	375.5 ± 115.7
Barn owl (A)	302.5 ± 66	387.5 ± 102.9	250 ± 48.9	243 ± 64.7
Barn owl (J)	155 ± 15.3	227.8 ± 45.3*	167.8 ± 24.4	169.1 ± 28.6
Alkaline phosphatase (U/L)				
Brahminy kite	20.5 ± 11.5	15.9 ± 11.4	22 ± 9.1	26.1 ± 6.9
Barn owl (A)	52 ± 22.4	51.5 ± 21	47.8 ± 19.6	55.8 ± 18.9
Barn owl (J)	181 ± 77.8	184.88 ± 75.9	142.1 ± 29.1	91.8 ± 40.8
Creatinine kinase (U/L)				
Brahminy kite	369.4 ± 254	506.8 ± 352.6	438.9 ± 398.6	343.6 ± 123.9
Barn owl (A)	1,749.3 ± 364.1	3,220.5 ± 907.2	1,901 ± 510.6	1,621.5 ± 291.6
Barn owl (J)	1,609.8 ± 241.5	3,172 ± 832.1*	2,047.3 ± 731.1	1,975.3 ± 1,409.6
Uric acid (mg/dL)				
Brahminy kite	6.3 ± 2.8	10.5 ± 8.2	8.7 ± 3	15.2 ± 10
Barn owl (A)	10.8 ± 1.4	9.4 ± 5.1	12.9 ± 5.9	18.9 ± 5.8
Barn owl (J)	11.9 ± 1.8	15.2 ± 6.7	10.8 ± 2.2	18.1 ± 5.8
Ammonia (mg/dL)				
Brahminy kite	40.5 ± 34.1	66.1 ± 26.2	66.1 ± 33.3	61.5 ± 45.7
Barn owl (A)	18.5 ± 23.3	14.8 ± 5.4	12.8 ± 12.4	47.3 ± 29.8
Barn owl (J)	37.9 ± 3.1	26 ± 20.6	34.8 ± 22.2	32.4 ± 39.1

Values are presented as mean ± SD.
*Values differ significantly from day 0 ($p < 0.05$).

Blood biochemistry showed that AST and CK increased in Brahminy kite and juvenile Barn owl groups and decreased slightly on day 7 and day 30. The increase in AST and CK levels was caused by skeletal muscle damage [18]. Therefore, the increasing values in this study are likely associated with muscle cell damage from the intramuscular injection rather than hepatic toxicity. A similar result was reported in the study of intramuscular meloxicam injection in the Japanese quail [19]. The creatinine level also increased in the early stages (day 1) in the Brahminy kite group. On the other hand, creatine is primarily excreted in the urine before being converted to creatinine. Therefore, plasma creatinine levels are low and do not accurately assess avian renal function [20]. Greater amounts of creatinine can differentiate between severe muscle damage and reduction of glomerular perfusion [21]. Thus, the increase in creatinine in this study was attributed to muscle damage in accordance with the elevations of AST and CK.

In conclusion, a single intramuscular injection of 1 mg/kg meloxicam in Brahminy kites and Barn owls was not associated with clinical morbidity, hepatotoxicity, gastrointestinal toxicity, and nephrotoxicity in the short- and long-term periods.

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