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First basic biochemical and physiological analysis of black kites during uttarayan: A comparison of injured versus healthy

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Abstract

Black kites are important part of our ecosystem. The number of Black kites (*Milvus migrans govinda*) in India is declining year on year, with destruction of nesting and feeding sites being linked as a possible cause. As such it is vitally important we preserve this species, and can efficiently and effectively treat those that are injured, to improve their welfare and prevent future decline in their numbers. The purpose of this study was to compare biochemical and haematological parameters as there is no data available for this species in literature from India. In this study we have performed the biochemical and haematological parameters on four groups of black kite birds Amputation kites (Group A), pox kites mostly young birds (Group B), Healthy releasable birds from kite string injury (Group C), Control group (Group D). Our results shows that SGPT level in group C was highest compared to other groups. ALP level was highest in group B. K, Ca and PO₄ level did not show any significant increase or decrease. RBC levels were significantly lower in group B compared to other groups. Total protein levels did not show any significant difference amongst the group. CpK level was highest in group B compared to the other groups. Uric acid level did not show any significant difference. This is the first study where we have done biochemical and physiological analysis injured and pox lesioned Black kites from India during the kite festival.

Keywords: Black kites, biochemistry, healthy, pox

1. Introduction

Raptors are excellent bioindicators of a healthy ecosystem. Literature had placed emphasis on blood as a health monitoring tool for wild birds (Dawson and Bortolotti, 1997; Newman *et al.*, 1997; Olsen *et al.*, 2001; Balbontin and Ferrer, 2002) [1-4]. Very little studies focused on the use of haematological parameters of birds for biomonitoring of environmental conditions. Physiological stress indicators such as haematological parameters could be useful to evaluate the effects of contaminants heavy metals on birds. Haematological parameters can be considered indicators of toxicity in bird's studies (Pushpendra Pathak and K. S. Rana, 2012) [5]. Black kites (*Milvus migrans govinda*) are opportunistic and scavenging raptors which adapted to urban landscape better than any other avian species often fall victim to kite string injuries during the International kite flying festival, which takes place annually during Uttarayan that is in January every year, in Ahmedabad, India. The injuries mostly consist of lacerations serving through proapatagial ligament, proapatagium, and upto muscle, fractures, feather damage and entanglements are also found commonly. Anesthesia is often required in order to assess and repair these injuries. This event presents a unique opportunity to perform clinical studies because of the large number of patients with similar injuries presenting in a short period of time. The number of Black kites in India is declining year on year, with destruction of nesting site, lack of food as dogs and pigs compete for food with kites infections like pox and tricomoniasis and man made disasters like uttarayan are being linked as a possible cause. As such it is vitally important we preserve this species, and can efficiently and effectively treat those that are injured, to improve their welfare and prevent future decline in their numbers. The purpose of this study was to compare biochemical and haematological parameters as there is no data available for this species in literature from India.

2. Materials and Methods

2.1 Blood sampling

Blood samples 3 mL for haematological and 4 ml for biochemical examination were taken from the right jugular vein of six birds from each group; these were collected using syringe-

needle assemblies. The samples were collected within 1 minute of capture to ensure that the levels of the monitored parameters. The heparinised blood was immediately centrifuged at $800 \times g$ at $4^\circ C$ for 10 minutes, and plasma samples were stored at $-80^\circ C$ in Eppendorf test tubes until the analyses were performed. The samples for haematological examination were collected in tubes with heparin and analysed immediately. Lesions of the pox were identified from the gross specimen studies. Differential leucocyte count was done from six representative samples from each group.

2.2. Biochemical Examinations

Selected plasma biochemical parameters like total protein, albumin, glucose, calcium, phosphorus, uric acid, aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured on the principle of photometric detection in a KONELAB 2 analyser using commercial test kits (Biovendor-Laboratorni medicina a.s., Brno, Czech Republic).

3. Results

Group A table 1, results shows the values of different biochemical parameters in six different birds in amputation kites. Group B, table 2, results shows the value of different biochemical parameters in birds with pox lesions mostly young birds. Group C, table 3, results shows the value of different biochemical parameters in Healthy releasable birds from kite string injury (Group C), Control group (Group D).

In group A (Table 1) SGPT is 10.5 ± 3.33 (U/l), ALP (U/l) 4.15 ± 0.26 , K (mmol/l) 4.15 ± 0.26 , Ca (gm/dl) 9.01 ± 0.12 , PO₄ (gm/dl) 3.41 ± 0.35 , RBC ($10^4/\mu$ l) 318.16 ± 14.16 , Total protein (g/dl) 4.53 ± 0.28 , CpK (IU/L) 183.16 ± 25.44 , Uric acids (mg/dl) 5.11 ± 0.58 .

In group B (Table 2) SGPT is U/l 9.25 ± 6.375 , ALP (U/l) 250.25 ± 109.87 , K (mmol/l) 3.4 ± 0.25 , Ca (gm/dl) 8.67 ± 0.17 , PO₄ (gm/dl) 3.37 ± 0.32 , RBC ($10^4/\mu$ l) 227.5 ± 76 , Total protein (g/dl) 2.98 ± 0.53 , CpK (IU/L) 216 ± 28.5 , Uric acids (mg/dl) 5.5 ± 1.25 .

In group (Table 3) C SGPT is (U/l) 26.25 ± 16.87 , ALP (U/l) 83.25 ± 14.25 , K (mmol/l) 3.625 ± 0.37 , Ca (gm/dl) 8.2 ± 0.3 , PO₄ (gm/dl), 3.37 ± 0.57 , RBC ($10^4/\mu$ l) 318.16 ± 14.16 , Total protein (g/dl) 3.77 ± 0.35 , CpK (IU/L) 186.25 ± 30.62 , Uric acids (mg/dl) 4.42 ± 1.93 .

In group D (Table 4) SGPT is (U/l) 14.66 ± 6.88 , ALP (U/l) 65.16 ± 13.11 , K (mmol/l) 3.96 ± 0.17 , Ca (gm/dl) 8.66 ± 0.66 , PO₄ (gm/dl) 3.37 ± 0.57 , RBC ($10^4/\mu$ l) 278.16 ± 21.83 , Total protein (g/dl) 3.81 ± 0.55 , CpK (IU/L) 169.33 ± 34.55 , Uric acids (mg/dl) 5.63 ± 1.62 .

In differential leucocyte count (Table 5) leucocytosis was higher in group B compared to the other groups.

The results shows that SGPT level in group C was highest compared to other groups. ALP level was highest in group B, K, Ca and PO₄ level did not show any significant increase or decrease, RBC levels were significantly lower in group B compared to other groups. Total protein levels did not show any significant difference amongst the group. CpK level was highest in group B compared to the other groups. Uric acid level did not show any significant difference.

4. Discussion

The study has been conducted in order to set the base level for the Kites. Plasma biochemical parameters changes have been

used as indicators of nutritional and health status in birds⁶(Artacho, Soto-Gamboa, Verdugo, & Nespolo, 2007). Measurement of plasma metabolites offers a rapid and reproducible method to study the metabolism and physiological state of free-living bird.

Table 5 explains the differential leucocyte counts in different groups in different representative samples where In B group with Pox lesions the percentage of leukocytosis was greater compared to other groups. Leucocytosis has been associated with infections and inflammatory conditions in literature.

Our report suggest that in all the groups the TPP was around the 3.2 to 4.95 g/dl. Total plasma protein (TPP) is an important parameter for evaluating the nutritional status in birds and, thus, can be useful in determining some infectious diseases (Harr, 2002)^[2].

Aspartate aminotransferase (AST) is best described as a very sensitive indicator of liver disease and muscle damage (Harr, 2002)^[7]. The values of AST in this study were lower to those described for other species⁸(Garcia, Hermosa, & Aguirre, 2010). This change in AST in this study may correlate to bird physiology or inflammatory processes that might occur in migratory birds (Calabuig, Ferrer, & Muriel, 2010)^[9].

Alkaline phosphatase (ALP) in group B was comparatively higher than the group A, C and D. The value of the enzyme in A, C and D were in accordance with the previous reports for birds (Garcia, Hermosa, & Aguirre, 2010;)^[10]. The enzyme is related to metabolic activity in the birds. The value of B group was slightly higher than the other group this may be due to pox infection. Calcium and phosphorous values have been found in accordance with the other literature in other birds in normal range (Elarhani, 2018)^[11]. Uric acid values were found in normal range in this study.

5. Conclusion

To our knowledge, this is the first study of its kind that performs a biochemical analysis in different injured groups in *Milvus migrans govinda* in India. Current results represent a contribution to understand the physiological adaptations that facilitate the use of different habitats in these species and for establishing reference physiological and biochemical values and for comparison within the same species, for different reason with regard to their use in future conservation efforts or other studies.

Future sex-specific and long-term studies are necessary to establish seasonal baseline physiological values for a better understanding of the mechanisms underlying variation in body condition that affects reproductive fitness and subsequent life events in these raptors.

Table 1: Group A-Birds with amputation

Parameters	Values
SGPT (U/l)	10.5 ± 3.33
ALP (U/l)	52.5 ± 23.83
K (mmol/l)	4.15 ± 0.26
Ca (gm/dl)	9.01 ± 0.12
PO ₄ (gm/dl)	3.41 ± 0.35
RBC ($10^4/\mu$ l)	318.16 ± 14.16
Uric acids (mg/dl)	5.11 ± 0.58
Total protein (g/dl)	4.53 ± 0.28
CpK (IU/L)	183.16 ± 25.44

Table 2: Group B -Birds with Pox lesions

Parameters	Values
SGPT (U/l)	9.25+/- 6.375
ALP (U/l)	250.25 +/-109.87
K (mmol/l)	3.4+/-0.25
Ca (gm/dl)	8.67 +/- 0.17
PO4 (gm/dl)	3.37 +/-0.32
RBC (10 ⁴ /μl)	227.5 +/-76
Uric acids (mg/dl)	5.5 +/-1.25
Total protein (g/dl)	2.98+/-0.53
CpK (IU/L)	216+/-28.5

Table 3: Group C-Healthy releasable birds from kite string injury

Parameters	Values
SGPT (U/l)	26.25 +/-16.87
ALP (U/l)	83.25 +/-14.25
K (mmol/l)	3.625 +/-0.37
Ca (gm/dl)	8.2 +/-0.3
PO4 (gm/dl)	3.85 +/-0.12
RBC ((10 ⁴ /μl)	340.5 +/-49
Uric acids (mg/dl)	4.42 +/-1.93
Total protein (g/dl)	3.77+/-0.35
CpK (IU/L)	186.25 +/-30.62

Table 4: Group D-Control group

Parameters	Values
SGPT (U/l)	14.66 +/-6.88
ALP (U/l)	65.16 +/-13.11
K (mmol/l)	3.96 +/-0.17
Ca (gm/dl)	8.66 +/-0.66
PO4 (gm/dl)	3.37 +/-0.57
RBC ((10 ⁴ /μl)	278.16 +/-21.83
Uric acids (mg/dl)	5.63 +/-1.62
Total protein(g/dl)	3.81 +/-0.55
CpK (IU/L)	169.33 +/-34.55

Table 5. Differential Leucocyte count of Different groups.

Sr. No	Group /Slide Code	H%	L %	E %	M %	B %	Total 100	Leucocyte Count on smear	Platelets	Cellular Morphology of RBCs
1	GroupA-1	66	31	1	2	0	100	adequate		Normal
2	A-2	73	24	2	1	0	100	adequate		Normal
3	A-3	71	24	2	2	1	100	adequate		Normal
4	A-4	71	26	2	1	0	100	adequate	Thrombocytosis	Normal
5	A-5	68	28	2	1	1	100	adequate		Normal
6	A-6	70	28	1	1	0	100			Normal
7	GroupB-1	77	20	1	2	0	100		Thrombocytosis	Normal
8	B-2	79	17	1	3	0	100	Leucocytosis		Normal
9	B-3	67	30	2	1	0	100	Leucocytosis	Thrombocytosis	Normal
10	B-4	74	24	0	1	1	100	Leucocytosis	Thrombocytosis	Normal
11	B-5	77	22	0	1	0	100	Leucocytosis		Normal
12	B-6	59	38	2	0	1	100			Normal
13	GroupC-1	69	28	1	2	0	100			Normal
14	C-2	58	40	0	2	0	100			Normal
15	C-3	49	50	0	1	0	100			Normal
16	C-4	54	43	1	2	0	100	Leucocytosis	Thrombocytosis	Normal
17	C-5	70	25	2	2	1	100			Normal
18	C-6	69	29	1	1	0	100			Normal
19	GroupD-1	78	22	0	0	0	100			Normal
20	D-2	71	26	2	0	1	100	Leucocytosis	Thrombocytosis	Normal
21	D-3	65	33	1	1	0	100	Leucocytosis	Thrombocytosis	Normal
22	D-4	77	21	1	1	0	100			Normal
23	D-5	73	25	0	1	1	100			Normal
24	D-6	70	27	2	1	0	100		Thrombocytosis	Normal

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