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Estimation of Haematobiochemical and Cortisol Level in Captive Elephants

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Abstract: Total number of 30 Asian Elephants were analysed for haematobiochemical and cortisol level in the different Tiger Reserves of Madhya Pradesh. The freshly laid feacal samples and blood were collected from each elephant. These biological samples were analyzed by using commercially available diagnostic kits and reagents. Haematobiochemical parameters showed correlation between high rise of neutrophils, TEC, TLC, SGOT/AST, SGPT/ALT, ALP, UA in elephants those have higher level of cortisol. Male and female showed no difference in level of cortisol however sub-adults showed higher level of cortisol as compare to calves and adults.

Index Terms: Asian Elephants, Tiger Reserve, Cortisol, Haematobiochemical analysis.

I. Introduction

The Asian elephant (*Elephas maximus*) enlisted as endangered species in the schedule – I A of the Wildlife (Protection) Act, 1972. In India, existing population of captive Asian elephants is around 14500 to 15300 while 44000 to 56000 populations found in free ranging conditions in and around the world (IUCN, 2008). According to evolutionary history, Asian elephants came into existence since more than 16 million years ago and known as the largest terrestrial mammal of the animal kingdom. The Asian elephants have been trained for multifaceted works in the National Parks and tiger reserves of India. Their potentials are being utilized to reach distant places where vehicle mobility is not possible for wildlife conservation related works on routine basis. An infectious and non-infectious disease induces the stress level owing to hypo/hyperactive endocrinal secretions which affect the immunity, agility and strength (Hing *et al*., 2016). The stress building factors may be evaluated by analysis of excretory-secretary hormones particularly cortisol which is found in faeces, urine, saliva and tears. The cortisol is a steroid hormone that is responsible for wide range of process within the body including immune response, regulation of metabolites acting as an anti-inflammatory and important for keeping active body condition (De Iudicibus *et al*., 2011). In park elephants, there are many factors responsible health conditions, and the infectious diseases are reported as one of the main causes for morbidity and mortality. The chronic condition of the diseases is capable to increase the stress level induced by endocrinal secretions that may reduce the potentials leading to weakness and exercise intolerance habits in park elephants (Paudel *et al*., 2016). Thus the estimation of haematobiochemical and cortisol analysis for stress evaluation is the need of hours.

II. Study area

a. Location

The study was conducted on tiger reserves of Madhya Pradesh viz. Kanha, Bandhavgarh and Panna tiger reserve as maintaining by the Forest Department under Ministry of Environment, Forest and Climate Change, Govt. of India. Laboratory investigations were carried out in the School of Wildlife Forensic and Health, Jabalpur.

b. Selection of animals

Blood and faecal samples were collected from 30 elephants including both the sex (male and female) and different age group (calves, sub-adults, and adults) of elephants with the help of mahout and wildlife veterinary physician.

III. Methodology

A. Haematological studies

a. Collection of blood samples:

The blood samples were collected from ear vein in sterile vacutainer containing EDTA (ethylene diamine tetra acetic acid) as an anticoagulant for haematological studies and without anticoagulant vacutainer (Bio In-Vitro Diagnostics Pvt. Ltd., Waghodia) for biochemical studies. The collected blood samples from each elephants were analyzed for haematological studies by using semi auto haematology analyzer (PG-6800 vet) and also done by manually. The serum of individual elephants was separated within 2-3 hours of blood collection and stored at 4°C prior to analysis. Serum samples were analyzed for liver function and kidney function parameters by using ERBA diagnostic kits and semi auto analyzer (ARK diagnostics, Mumbai) and interpretations of the findings was done in accordance to the guidelines of Benjamin (2013).

b. Serum biochemistry analysis:

Serum samples were subjected to monitor liver function and kidney function tests as mentioned below: Serum glutamic oxaloacetic transaminase/Aspartate amino transferase (SGOT/AST), Serum glutamic-pyruvic transaminase/Alanine amino transferase (SGPT/ALT), Alkaline phosphatase (ALP), Total protein (TP), Total bilirubin (TBIL) Serum creatinine (CRE), Blood urea nitrogen (BUN), Uric acid (UA) were assessed by following the kinetic as well as end point chemistry protocols using commercially available Erba diagnostic kits with the help of Semi-Auto analyzer (mini CHEM 100, APK Diagnostic System Pvt. Ltd.)

B. Faecal cortisol level analysis:

Faecal cortisol level was assessed by using commercially available ELISA diagnostic kits (Cortisol Enzyme Immunoassay Kit, Detect X, ARBOR ASSAYS) with the help of ELISA reader according to the manufacturer's instructions Calculation of analyzed samples:

Faecal solid (pg/gm) = Assay concentration (i.e. pg/ml) x assay dilution factor x reconstitution vol. (ml) ÷ evaporation vol. (ml) ÷ 0.1g faecal solid/ml

C. Statistical analysis of data

Statistical analysis of data was carried out by using standard statistical procedure and interpretations were based on the finding as suggested by Snedecor and Cochran (1967).

III. Results and discussion:

a. Heamatological analysis:

The haematological values are presented in the table 01. In the present study, observed mean values of TEC, TLC, Hb, HCT, MCH, MCHC, monocytes, lymphocytes, neutrophils in elephants examined from Kanha tiger reserve, Panna tiger reserve and Bandhavgarh tiger reserve. The observed mean values of TLC, TEC, Hb, HCT, MCH, MCHC, and DLC (monocytes, lymphocytes, neutrophils) were significantly higher ($p<0.05$) with the haematological parameters as recorded by Sarma (2011) in apparently healthy elephants (TEC, 2.5-5.0 million/µl, Hb, 11-15 gm/dl, HCT, 30-40%, MCH, 35-50 pg, MCHC, 25-40 g/dl, monocytes, 1-3%, lymphocyte, 40-60%, neutrophils, 22-50%). Haematological parameters of apparently healthy captive elephants were also studied by Jayamethakul (2017) and Anchal (2017) from different parts of country. Our findings are similar to them while significant increase in the mean value of TLC, TEC, Hb, HCT, MCH, MCHC, and DLC was noticed higher in sub-adults group of elephants. The significant increase in the haematocrit values in the present study may be associated with parasitic infestation as well as, elevation of the stress level in the park elephants. Wijesekera *et al*. (2018) have also studied heamatological parameters in blood flukes infected elephants with high rise of heamatological values. Similarly, Bhoyar *et al.* (2014) studied the effect of schistosomiasis in Asian elephants and observed high rise of eosinophils in the infected animals of *B. neiri*. Anchal (2017) recorded remarkable increase values of eosinophils in blood fluke infected elephants of different parks of Madhya Pradesh. Plausible reason for increasing the haematological values of elephants of Panna tiger reserve may be owing to occurrence of gastrointestinal parasitism (GIP) as well as physiological stress because in these elephants also recorded elevated cortisol level.

Table 01: Park wise heamatological analysis of blood samples of Elephants (Mean±SE)

S. No.	Parameters	Kanha tiger reserve (N=10)	Panna tiger reserve (N=10)	Bandhavgarh tiger reserve (N=10)	Total (N=30)	*p*-values
1.	TLC (x10³µl)	16.80^{B}±01.07	45.50^{A}±04.40	12.90^{B} ± 00.80	25.10 ± 03.08	*p* <0.00001 The result is significant at *p*<0.05
2.	TEC (x10⁶µl)	04.98^{B}±00.08	09.08^{A}±00.38	03.01^{B} ± 00.80	05.69 ± 00.22	
3.	HB (gm/dl)	13.48^{B}±00.36	23.28^{A}±01.73	11.50^{B} ± 00.26	16.08 ± 01.19	
4.	HCT (%)	41.42^{B}±01.41	59.47^{A}±04.82	35.04^{B}±01.03	45.31 ± 02.53	
5.	MCH (pg)	39.90^{B}±02.27	45.73^{A}±00.45	38.43^{B} ± 00.67	41.35±00.97	
6.	MCHC (g/dl)	32.89^{B}±00.93	39.35^{A}±00.62	32.19^{B} ± 00.38	34.81± 00.7	
7.	Monocytes %	07.00^{C}±00.37	10.00^{A}±00.81	06.70^{B} ± 00.98	07.70± 00.73	
8.	Lymphocytes%	63.2^{A} ±01.33	34.50^{B}±02.60	32.80^{B} ± 01.20	43.50 ± 02.70	
9.	Neutrophils %	28.70^{B}±00.39	69.30^{A}±02.20	59.10^{A} ± 09.40	52.03 ± 04.14	
10.	Eosinophils %	06.90^{B}±00.75	05.70^{A}±00.63	10.30^{A} ± 00.70	8.30± 00.77	
11.	MCV (fl) (NS)	122.70±07.83	116.40±02.68	121.85±02.85	120.31 ±02.86	Non-significant *p* >0.05
12.	PLT ($X10^{9}$/l) (NS)	311.30±26.50	286.8±21.40	374.40± 39.70	324.16 ±18.10	

$^{(A,B,C}$ Means with different superscript showing significant difference at *p*<0.05)

b. Serum Biochemical Examinations

In the present study observed mean values of serum biochemical parameters in elephants examined in this study from Kanha tiger reserve, Panna tiger reserve and Bandhavgarh tiger reserve. The observed mean values of ALT, AST, BUN and UA were significantly higher (p<0.05) with the biochemical parameters as recorded by Sarma (2011) in apparently healthy elephants (ALT, 1.5-3 µ/l, AST, 15-35 µ/l, BUN, 10-13 mg/dl, UA, 0.4-3.4 mg/dl). Biochemical parameters of apparently healthy captive elephants also studied by Wijesekera *et al.* (2008), Bhoyar *et al.* (2014), Singh *et al.* (2015), Jayamethakul (2017) and Anchal (2017) from different parts of country with similar range of values encountered in the present study. Reason for increasing the serum biochemical values of elephants of Panna tiger reserve may be owing to occurrence of gastrointestinal parasitism (GIP) as well as physiological stress as evidenced by elevated cortisol level. The biochemical values are presented in the table 02.

Table 02: Park wise biochemical analysis of blood samples of Elephants (Mean±SE)

S. No.	Parameters	Kanha tiger reserve (N=10)	Panna tiger reserve (N=10)	Bandhavgarh tiger reserve (N=10)	*p*-values
1.	ALT (µ/l)	09.71^{A} ± 00.48	05.84^{B}± 00.34	06.40^{B}± 00.48	*p*<0.00001 The result is significant at *p*<0.05
2.	AST (µ/l)	37.56^{A} ±01.70	42.44^{B}±01.6	43.40^{A}±02.75	
3.	Blood urea nitrogen (mg/dl)	11.98^{B}±00.56	15.70^{AB}±02.05	19.20^{A}±01.90	
4.	Uric acid (mg/dl)	4.83^{A} ±00.27	05.54^{AB}±00.16	04.29^{A} ±00.04	
5.	Alkaline phosphates (µ/l)	400^{A}.72±14.08	413^{A}.77±16.37	409.51^{B}±18.67	
6.	Total Protein (g/dl)	07.88±00.29	08.19±00.19	08.07±00.66	Non-significant *p*>0.05
7.	Creatinine (mg/dl)	00.84±00.27	00.48±00.07	01.50±00.07	
8.	Total bilirubin(mg/dl)	00.35±00.14	00.55±00.15	00.83±00.06	

$^{(A,B,C,}$ Means with different superscript showing significant difference at *p*<0.05)

c. Cortisol metabolites estimation from faecal samples of Elephants:

The mean cortisol metabolites were observed from Kanha tiger reserve, Panna tiger reserve and Bandhavgarh tiger reserve (234.2±27.4, 250.8±16.8 and 232.2±19.6 ng/gm) respectively (table 03). The level of cortisol metabolites were observed non-significant (*p*>0.05).In all the elephants of different tiger reserves of

Madhya Pradesh. Faecal cortisol metabolites have been identified as an index of stress in animals (Bayazit, 2009). The study showed that housing and nutritional management practices in different parks are similar with more or less, where seasonal variations may influence the adverse situation. Chichilichi *et al.* (2018) emphasize that the nutrients deficiency during dry season leading to elevated glucocorticoids in wild elephants. Subsequently cortisol levels found elevated in winter than rainy season implying that winter season have more stressful situation then rainy season. However, in the present study the samples were collected in the summer season and the results envisaged that non-significant yield of cortisol levels in elephants of different tiger reserves. The mean cortisol metabolites were observed in males and females (245.67±22.71 and 229.61±44.14 ng/gm) respectively, which was found non-significant. The study shows that sex wise difference may observe with the state of musth in males and during pregnancy in females. Ghosal *et al.* (2013) observe cortisol level decreases during musth conditions and they shows aggression due to suppression of testosterone hormones secretion by glucocorticoids. On the other hand, the cortisol levels increases during the advance pregnancy period may lead to indicator of pregnancy diagnosis. The mean cortisol metabolites were observed in calves, sub-adults and adults (189.99±92.96, 280.89±19.29 and 266.83±37.11 ng/gm) respectively. The level of cortisol metabolites showed significantly higher (p<0.05) in sub-adults and adults than to calves. Our findings were similar to Stead *et al*. (2000), who reported higher levels of glucocorticoids in juvenile African elephant kept in the small enclosures compared to large areas. However, park elephants in different tiger reserves are reared in semi captive habitats but sub-adults are confined to training them for wildlife conservations activities might be the factor for increase the stress levels compared to adults in the present study.

Table 03: Park and sex wise cortisol concentration of Elephants in different tiger reserves

S. No.	Tiger Reserve	(Mean±SE)		Total (N=30)
		Male	Female	
1.	Kanha Tiger Reserve (N=10)	265.33± 22.49	187.50± 56.21	234.20± 27.4
2.	Panna Tiger Reserve (N=10)	252.28± 22.32	262.00± 36.05	250.80 ± 16.80
3.	Bandhavgarh Tige Reserve (N=10)	219.42± 23.36	168.70± 76.80	232.20± 19.60
Total (N=30)		**245.67±22.71**	**229.61± 44.14**	**239.60± 12.20**

Non-significant, p<0.00001

V. Conclusion:

Haematobiochemical examination of captive elephants of Madhya Pradesh was revealed that elevated values of heamatological parameters and serum parameters showed high cortisol level. Stress is directly affected to health of the animal. That's why, proper management of captive elephants is very important for their health, which might be helpful for healthy environment and ecosystem.

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