

Original Research Article

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## Clinico–Physiological, Haemato–Biochemical and Urinalysis of 20 Adult Captive Indian Elephants (*Elephas maximus*)

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### ABSTRACT

Health is determined by biological, physiological and environmental variables. These variables differ for different species of animals. Species specific blood value reference intervals emphasising on variations associated with age, sex and geographical location are ideal for the proper diagnosis and treatment of a disease because species, age, sex, management, exercise and geographical location can all affect haematological values. The aim of this study was to establish a set of haematological, serum biochemical and urine chemistry reference values for captive Asian elephants of South India. Twenty apparently healthy adult captive Asian elephants (*Elephas maximus*) of either sex (10 male, 10 female) were selected at random for the study. Elephants that were neonates, pregnant, in lactation, in any disease condition and in musth were excluded. Selected elephants were subjected to clinical, physiological, haematological, serum biochemical and urine evaluation. The data acquired from the study were compared with universal standards in order to formulate baseline values. The results showed similarity in haematological, serum biochemical and urine parametrical values with that of previously published studies. There were significant difference in basophil count, band cell count, alanine aminotransferase and pH value (dipstick) between male and female group. The authors indicated that specific control conditions can be further incorporated to find the true values for the various parameters under observation.

#### Keywords

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### Introduction

Elephants are the largest terrestrial animals alive today. These giant herbivores are considered to be a part of Indian tradition from the early age. Unfortunately, their population is declining due to habitat loss, poaching and

increase in human population (Mikota and Kahn, 2000). In the conservation of these endangered species, veterinarians play a pivotal role by monitoring their health in captivity and in wild. Knowledge of the normal physiological, haematological, serum biochemical and urine parameters of elephants are useful in evaluating their health status as

well as in monitoring the course of a disease or the response to a treatment. Reports on the normal parameters of elephants are few and reports on wild or free ranging elephants are even less common. This is due to the very few opportunities for collection of significant number of samples from captive, wild or free ranging animals. Elephant urine analysis is one of the less explored arena compared to haematology and serum biochemistry of elephants. Despite of the confirmation that elephants develop urinary tract infections, data regarding the same is very limited.

Urine collection and evaluation being least invasive technique, urine analysis holds a good potential and diagnostic value for monitoring of the general health of elephants. This article aims to establish baseline values for haematology, serum biochemistry and urine chemistry in clinically normal adult male and female elephants of South India.

## **Materials and Methods**

Twenty apparently healthy adult captive Asian elephants (*Elephas maximus*) of either sex (10 male, 10 female) were selected for the study at random. Elephants which were neonates, pregnant, in lactation, in musth, in any apparent disease condition were exempted from the study. The selected animals were subjected to clinical, physiological, haematological, serum biochemical and urine evaluation.

**Clinical and Physiological evaluation:** Observations were made about the general appearance, behavioural habit, feeding habit and voiding habit (urine and dung) of the animals. Physiological parameters such as rectal temperature, pulse rate and respiratory rate were observed for a duration of one minute and recorded. **Blood collection:** Animals were placed in lateral recumbency. Blood was drawn from superficial marginal

vein located at the external aspect of the ear using a clean, sterile 16-gauge disposable vacutainer needle into a sterile vacutainer tubes under aseptic conditions.

**Haematology evaluation:** Blood samples were collected in 2ml EDTA vacutainer tubes for the evaluation of haematological parameters (Orphee mythic 18vet - analyser) viz. haematocrit (HCT), haemoglobin (Hb), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocyte count, total leukocyte count (TLC), differential leukocyte count (DLC), platelet count (PC) and mean platelet count (MPC) on the day of presentation.

**Serum biochemical evaluation:** Blood samples were collected in 4ml serum vacutainer tube for the evaluation of serum biochemical parameters (Master T, Hospitex diagnostics, Italy) viz. blood urea nitrogen (BUN), serum creatinine, total bilirubin, direct bilirubin, indirect bilirubin, glucose, total protein, albumin, globulin, albumin globulin ratio, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) on the day of presentation.

**Urine Collection:** For ease of sample collection, urine samples were collected from elephants at dawn in the presence of mahout under sterile conditions. Mid-stream urine (100 ml) was collected in sterile urine collection vial. **Urinalysis:** Urine was evaluated for colour, appearance, osmolality, mucous threads, pH (Cyberscan pH510 meter), protein (sulfosalicylic acid - SSA Test), and leukocytes, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketone, bilirubin, glucose using (Mission urine reagent strip from ACON LABS INC - dipstick).

**Statistical analysis:** The categorical variables

were summarized in frequency (n) and percentage (%) and the continuous ones were expressed in mean and standard error (Mean  $\pm$  SE). In order to compare quantitative variables in the groups, independent sample t test for normally distributed variables and Mann Whitney U test for non-parametric variables were performed. The association between urine colour, appearance and presence of mucus threads among different sex were assessed using the Chi square test and Fischer's exact test appropriately. The statistical analysis was carried out using IBM SPSS version 25.

## Results and Discussion

The clinical examination (Table 1) was performed at the time of sample collection. All the animals included in the study were clinically normal and did not show any signs of systemic disorder or infection. All animals were healthy with good skin condition and normal pink mucous membranes. The animals were active and alert, with normal feed and water intake. There were no abnormalities detected in the frequency, colour and consistency of urination and defaecation. Physiological parameters like rectal temperature, pulse rate and respiration rate were collected on the day of presentation and evaluated. The values acquired were statistically analysed separately for male and female elephants. The mean, standard error of mean and t- test significance for the various parameters were tabulated (Table 2). The values obtained were compared with the previously published reports and found to be in accordance with (Buss and Wallner, 1965; Kock *et al.*, 1993; Mikota, 2006). The variations observed were within normal range for the particular sex and age group.

Blood samples were collected for haematological and serum biochemical analysis on the day of presentation and evaluated. The values obtained were

statistically analysed separately for male and female elephants. The mean and standard error of mean and t-test significance for the various parameters were tabulated (Table 3 and 4). The Haematocrit (HCT), Hb, RBC, MCV, MCH, MCHC, RDW, Platelet count values obtained for both male and female elephants were in agreement with the studies conducted by Brown and White, 1979; Salakij *et al.*, 2005; Klinhom *et al.*, 2017). In the present study, the bilobed monocytes were greater in number than the number of lymphocytes which was in accordance with the findings of Salakij *et al.*, (2005). Reticulocytes were completely absent as observed previously by (Salakij *et al.*, 2005; Klinhom *et al.*, 2017). However, WBC count was found to be relatively high and this may be attributable to the variations arising from the different geographical locations where the elephants are held and subsequent variable management conditions (Klinhom *et al.*, 2017). The number of eosinophils were more than monocytes as observed previously by (Salakij *et al.*, 2005; Mel *et al.*, 2014) but was found to be in contrast with the studies conducted by (Dastjerdi *et al.*, 2016; Klinhom *et al.*, 2017). Mild variations in the values may also be attributed to the different laboratory conditions, blood collection techniques and storage conditions. All the haematological parameters considered had no significance difference between the male and female group, except for basophils and band cells which showed significant difference between male and female group.

The BUN and total bilirubin value obtained were in agreement with the values reported by (Silva and Kuruwita, 1993; Allwin *et al.*, 2015). The high total bilirubin values for elephants may be due the absence of gall bladder which leads to continuous secretion of bile from hepatic tissues. Creatinine values obtained were found to be in the upper limit of normal range obtained previously by other

authors (Nirmalan and Nair, 1971; Fowler, 1986; Silva and Kuruwita, 1993). This variation may be influenced by the amount of creatinine generated by the skeletal muscles (Osborne *et al.*, 1972). The serum glucose values can be inclined with respect to the age advancement and nutrition of the animal, overall mean of glucose values however was in accordance with previously published reports (Fowler, 1986; Mikota *et al.*, 1994).

Brown and white, (1980) had opined that age advancement could be a probable reason for high serum protein and increase in globulin which could increase the viscosity of the blood. This can be a reason for the high protein values obtained in the present study, although the values lie within the previously reported acceptable range. Age, sex or musth has no effect on the A/G ratio in Asian elephants as reported by (Pc, 1994).

The serum cholesterol values obtained are in agreement with those reported by Wallach and Boever (1983). The ALP, AST and ALT values obtained were found to be within the accepted reference range obtained in earlier studies (Mikota, 2006; Pandit *et al.*, 2015).

Urine samples were collected on the day of presentation and evaluated. The values acquired were statistically analysed separately for male and female elephants. The mean and standard error of mean and t-test significance for the various parameters were tabulated (Table 5).

The appearance and colour of the urine were variable from straw coloured to dark yellow and from clear to cloudy. Turbidity or cloudiness in the urine is due to the presence of mucus cells and crystals in the urine (Benedict, 1936; Simon, 1958). Specific gravity values in the present study is in agreement with previous reports (Benedict *et*

*al.*, 1936; Wiedner *et al.*, 2009). Healthy elephant kidney has negligible number of renal papillae and the contact between the urine and outer medulla at the calyx is limited, which suggests that elephant kidney has decreased ability to concentrate urine compared to other species (Schulte, 1937; Maluf, 1995). The osmolality values obtained in the present study was found in accordance with values obtained by (Wiedner *et al.*, 2009). Wiedner *et al.*, (2009) reported that the urine osmolality in elephants is considerably low when compared to other domesticated species.

The urinary pH of elephant is found to have inclination towards alkalinity like other herbivores (Benedict *et al.*, 1936). The values obtained in pH meter and dipstick was found to be in the alkaline range and there was no significant difference noted between pH values in pH meter and dipstick as observed using t- test, which was found in accordance with previous report (Wiedner *et al.*, 2009). Presence of glucose, ketone, leukocytes, blood and protein is nil to negligible.

Protein evaluated using sulfosalicylic acid - SSA test and dipstick showed similar values. Urobilinogen and bilirubin values were observed in traces. Osborne *et al.*, (1999) in his study found that minor amounts of bilirubin can be a normal finding in healthy elephants. The variations observed in serum biochemical values were within normal range for the particular sex and age group.

Health is determined by biological, physiological and environmental variables. These variables differ for different species of animals. Since haematology, serum biochemistry and urinalysis parameters has the potential to indicate the physiology of the body, these values can be used for assessing the health of the animals.

**Table.1** Observations on clinical examination of the animals

Sl. No.	Parameter	Unit	Observation	
			Male	Female
1	General Appearance	Obese, Normal, Emaciated	Normal	Normal
2	Behavioural Habit	Active, Dullness, Abnormal behaviour	Active, Alert	Active, Alert
3	Feeding Habit	Frequency, Quantity, Duration	No Abnormalities Detected	No Abnormalities Detected
4	Water Intake	Frequency, Quantity, Duration	No Abnormalities Detected	No Abnormalities Detected
5	Dung Voiding Habit	Frequency, Quantity, Consistency, Colour, Smell	No Abnormalities Detected	No Abnormalities Detected
6	Urine Voiding Habit	Frequency, Duration, Quantity, Flow, Colour, Smell	No Abnormalities Detected	No Abnormalities Detected

**Table.2** Observations on physiological parameters

Sl. No.	Parameter	Unit	Observation	
			Male	Female
1	Rectal Temperature	°F	96.65±0.07	96.89±0.06
2	Pulse rate	Pulse/min	30.90±1.19	29.80±0.85
3	Respiratory rate	Breath/min	5.1±0.27	4.9±0.27

**Table.3** Observations on haematological parameters

Sl. No.	Parameters	Unit	Observation		P value
			Male	Female	
1	Haematocrit	%	31.07±0.81	30.29±0.53	0.44
2	Haemoglobin	g/dL	10.92±0.42	11.01±0.42	0.88
3	Total Erythrocyte Count	10 <sup>12</sup> /L	02.62±0.08	02.37±0.08	0.66
4	Mean Corpuscular Volume	fL	119.51±5.14	129.03±5.20	0.21
5	Mean Corpuscular Haemoglobin	pg	41.94±1.85	46.64±1.78	0.08
6	Mean Corpuscular Haemoglobin Concentration	g/dL	35.52±1.98	36.63±1.92	0.69
7	Red Cell Distribution Width	%	14.98±0.19	15.18±0.25	0.54
8	Reticulocyte	%	00.00±0.00	00.00±0.00	0.00
9	Total Leukocyte Count	10 <sup>9</sup> /L	17.61±0.21	17.20±0.27	0.26
10	Bands	10 <sup>9</sup> /L	0.047±0.008	0.075±0.008	0.03
11	Neutrophils	10 <sup>9</sup> /L	05.38±0.25	04.97±0.13	0.18
12	Eosinophils	10 <sup>9</sup> /L	00.47±0.10	00.69±0.07	0.12
13	Basophils	10 <sup>9</sup> /L	0.065±0.006	0.033±0.008	0.01*
14	Lymphocytes	10 <sup>9</sup> /L	04.96±0.31	05.06±0.36	0.84
15	Bilobed Monocytes	10 <sup>9</sup> /L	06.30±0.12	06.02±0.17	0.22
16	Monocytes	10 <sup>9</sup> /L	00.41±0.10	00.34±0.07	0.20
17	Bands	%	00.26±0.04	00.43±0.05	0.03*
18	Neutrophils	%	30.55±1.35	28.93±0.68	0.31
19	Eosinophils	%	02.72±0.58	03.97±0.42	0.10
20	Basophils	%	00.36±0.038	00.18±0.04	0.01*
21	Lymphocytes	%	28.15±1.72	29.29±1.79	0.65
22	Bilobed Monocytes	%	35.81±0.80	35.16±1.32	0.22
23	Monocytes	%	03.48±0.58	02.00±0.46	0.20
24	Platelet Count	10 <sup>11</sup> /L	03.48±0.06	03.46±0.11	0.90
25	Mean Platelet Volume	fL	04.47±0.08	04.66±0.09	0.16

N.B: P value derived from independent t-test; \* indicates significant difference; Bands and basophil

**Table.4** Observations on serum biochemical parameters

Sl. No.	Parameters	Unit	Observation		P value
			Male	Female	
1	Blood Urea Nitrogen	mg/dL	15.59±0.21	15.71±0.24	0.70
2	Creatinine	mg/dL	01.64±0.09	01.41±0.11	0.13
3	Total Bilirubin	mg/dL	00.72±0.02	00.71±0.02	0.66
4	Direct Bilirubin	mg/dL	00.62±0.01	00.60±0.02	0.49
5	Indirect Bilirubin	mg/dL	0.103±0.015	0.108±0.009	0.35
6	Glucose	mg/dL	79.69±0.64	79.96±0.74	0.79
7	Total Protein	g/dL	07.07±0.13	07.08±0.13	0.94
8	Albumin	g/dL	02.51±0.10	02.56±0.08	0.73
9	Globulin	g/dL	04.55±0.07	04.52±0.09	0.80
10	A/G Ratio	-	00.54±0.02	00.56±0.02	0.66
11	Cholesterol	mg/dL	58.38±1.16	59.06±1.16	0.67
12	Aspartate Aminotransferase	IU/L	30.20±2.05	28.20±2.15	0.51
13	Alanine Aminotransferase	IU/L	03.17±0.17	02.49±0.21	0.03*
14	Alkaline Phosphatase	IU/L	276.0±32.96	247.3±24.70	0.36

N.B: P value derived from independent t-test; \* indicates significant difference in ALT value

**Table.5** Observations on urine analysis

Sl. No.	Parameters	Unit	Observation		P value
			Males	Females	
1	Colour	Deep Yellow	20%	50%	0.16
		Straw Yellow	80%	50%	
2	Appearance	Clear Urine	80%	40%	0.07
		Cloudy Urine	20%	60%	
3	Osmolality	mOsm/kg	628.04±0.40	517.15±1.70	0.11
4	Mucous Threads	Positive	0%	20%	0.14
		Negative	100%	80%	
5	pH Meter	-	6.81±0.19	7.53±0.27	0.05
6	Protein (Sulfosalicylic Acid)	Positive	0%	0%	-
		Negative	100%	100%	-
7	Leukocytes (Dipstick)	Leu/ µL	15±	15±	-
8	Nitrite (Dipstick)	Positive	50%	100%	-
		Negative	50%	0%	-
9	Urobilinogen (Dipstick)	mg/dL	0.20±18	0.20±18	1.00
10	Protein (Dipstick)	mg/dL	0.00±0.00	0.00±0.00	0.00
11	pH (Dipstick)	-	6.85±0.18	7.55±0.26	0.04*
12	Blood (Dipstick)	Ery/µL	0.00±0.00	0.00±0.00	0.00
13	Specific Gravity (Dipstick)	-	1.016±0.016	1.018±0.018	0.30
14	Ketone (Dipstick)	mg/dL	0.00±0.00	0.00±0.00	0.00
15	Bilirubin (Dipstick)	mg/dL	0.10±0.10	0.00±0.00	0.00
16	Glucose (Dipstick)	mg/dL	0.00±0.00	0.00±0.00	0.00

N.B: P value derived from independent t-test; \* indicates significant difference in pH dipstick value

The present study aims to furnish insight the physiological, haematological, serum biochemical and urine evaluation of normal adult captive Indian elephants (*Elephas maximus*) of South India with an objective to improve the preventive health care system of elephants. There were significant difference in basophil count, band cell count, alanine aminotransferase and pH value (dipstick) between male and female group. The authors indicated that specific control conditions like uniform age group, location, physical activity, feeding and watering habits can be further incorporated to find the true values for the various parameters under observation. Evaluation and understanding of these parameters can greatly contribute to early diagnosis of disease process and improve quality of treatment provided.

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