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Serum Biochemical Profile of Captive Asian Elephants (*Elephas maximus*) in Tamil Nadu, India

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ABSTRACT

The experiment was carried out in 46 apparently health captive elephants maintained in different parts of Tamil Nadu, India during the period from August 2020 to April 2021 to assess and evolve the baseline reference values for serum biochemical parameters in captive Asian elephants raised in the Tamil Nadu state of India.As part of the periodical health assessment, blood samples were collected from auricular vein for 46 apparently healthy captive Asian elephants maintained in Tamil Nadu, India. Biochemical analyses were performed using auto biochemical analyzer using commercial kits as per the recommendations of the manufacturer. The mean±SE, lower and upper value, 95% and 99% of confidence interval of the serum biochemistry parameters comprised of glucose, total protein, albumin, globulin, cholesterol, blood urea nitrogen (BUN), creatinine, total bilirubin, direct bilirubin, calcium, phosphorus, and magnesium, serum enzymes AST, ALT, ALP, CK, GGT and LDH were evaluated. The observed values were found to be within the normal ranges reported for the captive elephants and there were no significant differences observed between the age groups. These serum biochemical values would serve as baseline reference to evaluate the health and clinical conditions of captive elephant populations, as well as for planning health care measures in elephants of this region and adjoining areas.

KEYWORDS: Asian elephant, lactate dehydrogenase, serum biochemical parameters

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1. INTRODUCTION

Captive Asian elephants have been very closely associated and deeply entwined with the religion, myths and cultural heritage for centuries (Kumar et al., 2019). Elephants have adapted to a wide variety of environments in captive conditions. The health status of captive elephants depends on various factors including body mass index, husbandry management, nutrition and infectious and non-infectious diseases (Mikota et al., 1994, Schmitt, 1998, Sadler, 2001). Changes in environmental, social, physical, physiological and psychological factors in captive conditions can modify the homeostasis of individual elephants and lead to a range of health issues that could contribute to significant morbidity and mortality in elephants (Fowler and Mikota, 2006). Studies on captive elephant health and diseases are far less and hence evolving remedial measures to promote health and welfare remains challenging one. Understanding the health-related parameters and evidence of diseases in captive-reared elephants will be significantly helpful towards enriching the management as well as their healthcare (Veasey, 2006; Veeraselvam et al., 2021). The diagnostic and prognostic value of health parameters varies among domestic species and not all have been adequately studied in elephants (Fowler and Mikota, 2006, Janyamethakul et al., 2017). This study addresses significant knowledge gaps regarding health and disease management of elephants in captive situation.

The health and disease related information in captive elephants is still in a state of infancy in India. Elephants are prone to a variety of infectious and non-infectious diseases, but recognizing that they are even sick may be difficult and challenging (Miller et al., 2015; Miller and Fowler, 2015). Elephants often do not manifest clinical signs of illness until disease is well advanced. Such masking of clinical signs makes identifying and treating diseases in elephants is very challenging for veterinarians and zoo managers (Thapa et al., 2021). Clinical pathological examination including haematology, serum biochemistry and electrolyte values are most often the valuable diagnostic tools for elephants and are used to help differentiate diseases from healthy individuals, especially when clinical signs were nonspecific and other diagnostic techniques may not be available for immediate applications (Salakij et al., 2005; Fowler and Mikota, 2006).

Blood values especially haematology and serum biochemical values have been used in health assessments for a range of wild animals and so can be an essential part of investigating the responses to treatment, prognosis and management strategies (Girling et al., 2015; Edwards et al., 2020). Haematology and Biochemical values may differ according to the environmental circumstances of the area where the animal lives (Elarabany, 2018). Knowledge about region specific blood values represents an important prerequisite for the application of haematological and biochemical analyses in the evaluation of animal welfare in health and disease (Santos et al., 2020; Steyrer et al., 2021). The health profile of captive elephants depends on various factors including body mass index, husbandry management, geographic location and diseases (Mikota, 1994, Sadler, 2001). Factors such as gender, age, season and animal nutrition may also affect haematological and serum biochemical parameters (Morfeld and Brown, 2017; Chusyd et al., 2021). Furthermore, most of the blood parameter data for Asian elephants were available from different geographical locations and hence may not be relevant across all elephant population, due to influencing factors such as feeding management, geographic, climate and management conditions which affects the values (Janyamethakul et al., 2017). Serum chemistry values may also be altered by physiological factors such body condition, restraining methods, drugs used, nutrition and infectious and non-infectious diseases etc. (Watson et al., 1990; Norkaew et al., 2018; Thapa et al., 2021).Stress due to daily duty and activity, clinical condition, temperature, sex can make significant differences in blood values (Shawaf et al., 2018; Steyrer et al., 2021). Although normal serum biochemical reference values exist for Asiatic elephants, the overall range tends to be very broad (Dhairykar et al., 2022). Baseline samples obtained on individual captive elephants while healthy may provide the most accurate reference values when illness is suspected (Boonprasert et al., 2021).

Hence, this study was undertaken to establish the base-line serum biochemical profile for the captive elephants of Tamil Nadu state of India. This base-line serum biochemical data will be significantly useful not only for diagnosing and treating, but also for monitoring purposes by veterinarians, wildlife stake holders and policy makers and to formulate effective management and disease prevention measures.

2. MATERIALS AND METHODS

The study was carried out in 46 apparently health captive elephants maintained in different parts of Tamil Nadu during the period from August 2020 to April 2021.

The study population consisted of 46 female captive elephants maintained in different parts of Tamil Nadu. Out of this, 24 captive Asian elephants were assessed during a health and welfare campaign for captive elephants. Remaining 22 captive elephants were privately maintained elephants and were assessed during their periodical health and veterinary examinations. The age of elephants in this study ranged from 11 years to 62 years and all the elephants were apparently healthy during the study period. During sampling, the animals were positioned into lateral

recumbency with the help of their care takers and blood was collected from auricular vein Into plain vacutainer tubes and they were allowed to clot for 30 minutes at room temperature. Serum was separated by centrifugation at 2000 rpm for 10 min and the serum was stored at -20°C until further processing.

Serum biochemical examinations including organ function profiles such as blood urea nitrogen (BUN), creatinine, total bilirubin, direct bilirubin, blood metabolic profile such as total protein, albumin, globulin, blood glucose, serum cholesterol, calcium and phosphorus and serum enzyme profile such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were estimated in this study. Biochemical analyses were performed using auto biochemical analyzer (Selectra Pro XS Automatic Biochemistry Analyser, ELITECH, Netherlands) using commercial kits as per the recommendations of the manufacturer. Basic statistics including mean, median, and standard deviation were determined for each variable. For normally distributed variables, a 95% and 99%confidence interval for means was calculated. The statistical analysis of data was carried out using IBM® SPSS® ver.23.0 for windows.

3. RESULTS AND DISCUSSION

The mean±SE, lower and upper value, 95% and 99% of confidence interval of the serum biochemistry parameters are presented in Table 1. The parameters comprised of glucose, total protein, albumin, globulin, cholesterol, blood urea nitrogen (BUN), creatinine, total bilirubin, direct bilirubin, calcium, phosphorus, and magnesium, serum enzymes AST, ALT, ALP, CK, GGT and LDH were evaluated. The observed values were found to be within the normal ranges reported for the captive elephants and there were no significant differences observed between the age groups (Table 2 and 3).

3.1. Serum glucose

In the present study the overall mean value of serum glucose was 94.09±5.04 mg dl^{-1} with the range from 27 mg dl^{-1} to 232 mg dl^{-1}. This mean value of glucose was within the reference values reported for elephants by Fowler and Mikota (2006) who observed of a range of 60-116 mg dl^{-1}. The wide range of serum glucose levels observed could be due to the variations in feeding pattern, nutrition and age advancement of the elephants (Morfeld and Brown, 2017). Higher range values were considered to be associated with stress and due to the pain during restraining and blood collection in elephants (Fowler and Mikota, 2006). In this

Table 1: The Mean±SE, lower and upper value and confidence intervals (95% and 99%) for serum biochemistry values

Parameters	Mean±SE	Max	Min	95% confidence interval	99% confidence interval
Glucose (mg dl^{-1})	94.09±5.04	232	27	84.226–21.622	81.129–107.031
Totalprotein (g dl^{-1})	8.41±0.17	9.87	4.70	8.076–8.724	7.975–8.825
Albumin (g dl^{-1})	2.44±0.11	4.89	1.6	2.220–2.660	2.151–2.729
Globulin (g dl^{-1})	5.99±0.21	8	2.17	5.737–6.563	5.607–6.693
BUN (mg dl^{-1})	19.18±1.28	44	4.1	16.738–21.622	15.971–22.389
Creatinine (mg dl^{-1})	1.19±0.12	4.46	0.38	0.959–1.421	0.886–1.494
Cholesterol (mg dl^{-1})	48.63±2.82	146	29	43.096–54.164	41.357–55.903
ALT (U l^{-1})	7.09±0.50	16	3	6.092–8.068	5.781–8.379
AST (U l^{-1})	25.11±1.80	83	9	21.569–28.631	20.459–29.741
ALP (U l^{-1})	281.02±12.35	428	88	256.806–305.234	249.198–312.842
CK (U l^{-1})	182.69±12.92	460	64.4	161.248–211.912	153.288–219.872
GGT (U l^{-1})	19.78±0.97	41	10	17.881–21.679	17.285–22.275
LDH (U l^{-1})	352.41±12.03	600	184	328.832–375.988	321.423–383.397
Totalbilirubin (mg dl^{-1})	0.20±0.01	0.36	0.09	0.183–0.217	0.177–0.223
Directbilirubin (mg dl^{-1})	0.26±0.03	1.58	0.12	0.189–0.311	0.170–0.330
Calcium (mg dl^{-1})	11.77±0.25	16.75	7.8	11.288–12.240	11.130–12.390
Phosphorus (mg dl^{-1})	5.31±0.16	7.99	3.25	4.985–5.615	4.886–5.714
Magnesium (mg dl^{-1})	1.97±0.09	2.43	1.27	1.813–1.987	1.726–2.014
(Not significant - p>0.05)					

Table 2: Comparison of serum biochemistry parameters with age group of elephants

Groups	Glucose (mg dl^{-1})	Total protein (mg dl^{-1})	Albumin (m dl^{-1})	Globulin (m dl^{-1})	BUN (mg dl^{-1})	Creatinine (mg dl^{-1})	Cholesterol (mg dl^{-1})	Total bilirubin (mg dl^{-1})	Direct bilirubin (mg dl^{-1})
10–25 years (n=9)	96.11± 15.86	8.82± 0.57	2.32± 0.69	6.49± 0.84	14.90± 8.02	1.07± 0.21	46.00± 9.39	0.22± 0.07	0.23± 0.08
26–40 years (n=20)	87.85± 33.20	8.39± 1.26	2.43± 0.90	6.01± 1.52	19.78± 7.98	1.11± 0.53	51.10± 23.60	0.20± 0.05	0.29± 0.31
>40 years (n=17)	100.35± 41.90	8.20± 1.15	2.51± 0.64	5.71± 1.54	20.72± 8.93	1.35± 1.17	47.11± 17.51	0.19± 0.05	0.22± 0.07
f-Value	0.062	0.896	0.173	0.886	1.523	0.530	0.294	0.479	0.596
p Value	0.540	0.415	0.841	0.419	0.229	0.591	0.746	0.622	0.555

(Not significant - $p>0.05$)

Table 3: Comparison of serum biochemistry parameters with age group of elephants

Groups	ALT (Ul^{-1})	AST (Ul^{-1})	ALP (Ul^{-1})	CK (Ul^{-1})	GGT (Ul^{-1})	LDH (Ul^{-1})	Calcium (mg dl^{-1})	Phosphorus (mg dl^{-1})	Magnesium (mg dl^{-1})
10–25 years (n=9)	6.22± 1.71	31.44± 0.96	290.00± 93.64	226.85± 93.37	20.33± 8.27	313.44± 88.16	11.83± 1.30	5.51± 0.86	1.94± 0.29
26–40 years (n=20)	7.20± 3.67	23.40± 0.59	256.60± 78.96	176.50± 102.77	20.45± 5.98	356.35± 60.48	11.69± 1.36	5.34± 1.11	1.93± 0.15
>40 years (n=17)	7.41± 3.85	23.76± 9.18	305.21± 80.95	166.57± 57.33	18.70± 6.53	368.41± 96.68	11.81± 2.16	5.16± 1.18	1.95± 0.41
f-Value	0.363	1.544	1.642	1.513	0.352	1.401	0.032	0.319	0.004
p Value	0.697	0.225	0.205	0.231	0.705	0.257	0.967	0.728	0.995

(Not significant - $p>0.05$)

study the estimated values were higher when compared to the values (52–67 mg dl^{-1}) reported by Nirmalan and Nair (1969). A value of 119–131 mg dl^{-1} from elephants in Nepal was reported. Those variations might be occurring due to the differences in management and feeding pattern in different regions of the elephant range countries. The normal value of the glucose observed in this study indicated the wellbeing of the elephants in this study.

3.2. Serum total protein and albumin

Protein and albumin are essentially monitored in health evaluations. In this study, elephants had a mean value of 8.41±0.17 g dl^{-1} of total protein with a range from 4.70 g dl^{-1} to 9.87 g dl^{-1}, they had a mean value of 2.44±0.11 mg dl^{-1} of albumin with a range from 1.6 to 4.89 mg dl^{-1}. They had a median value of 5.99±0.21 g dl^{-1} globulin with a range from 2.17 to 8.0 mg dl^{-1}. No significant differences could be observed between the age groups. The mean value of total protein described by Silva and Kuruwita (1993) in free ranging Asian elephants was 8.4±1.2 g dl^{-1}, Janyamethakul et al. (2017) observed in captive Asian elephants a value of 6.5 to 8.9 g dl^{-1}. These values were similar to the values observed in this study.

Santos et al. (2020) reported that the food quality and quantity influenced the elephant's protein intake. Further, it was observed that during the dry season, elephants ingested low levels of protein and in wet seasons they consumed higher protein levels. The estimated values of total protein and globulin were observed to be higher than the values of other domestic animals like cattle (Alberghina et al., 2011) and horse (Riondet al., 2009). Fowler and Mikota (2006) opined that elephant had a higher total protein and globulin values and a lower albumin level than most of the other mammals, they further stated that the viscosity of elephant blood is almost twice that of other domestic species and this could be a probable reason for high serum protein and increase in globulin levels. The normal value of the protein observed in the study again indicated the wellness of these elephants.

3.3. Blood urea nitrogen and creatinine

BUN and creatinine are part of health evaluations in various species. The overall mean value of BUN in this study was 19.18±1.28 mg dl^{-1} and that of creatinine was 1.19±0.12

mg dl^{-1}. The BUN and creatinine levels were similar to the values (BUN- 5 to 20 mg dl^{-1}, Creatinine -1.0 to 2.0 mg dl^{-1}) reported by Fowler and Mikota (2006), however, these BUN values of this study were higher than the values (10±3.5 mg dl^{-1}) reported by Silva and Kuruwita (1993). The differences may also be related to the amount of protein in the diet and their metabolism in elephants which results in urea excretion (Santos et al., 2020). In this study, the range of BUN and creatinine values were 4.1 to 44 mg dl^{-1} and 0.38 to 4.46 mg dl^{-1} respectively. Though the upper range values observed were seems to be higher, the elephants were apparently healthy and no appreciable signs of any disease were evident. Wide range of BUN and creatinine values were found to be influenced by age of animals and season of sample collection. Senthilkumar et al. (1999) observed that young elephants had a lower value of urea and creatinine while it was higher in adults. This is might be due to the increased protein intake with the increased age of animals. The protein metabolism was found to be in higher level in adult animals than young ones (Rahman et al., 2018). Such wide ranges of BUN and creatinine levels may also reflect the impact of factors such as dehydration, varied protein intake, protein catabolism and renal excretions. In this study, there were no significant differences could be observed between the age groups of elephants. Santos et al. (2020) observed of seasonal changes in BUN levels in Asian elephants, which was found to be decreased (29.9%) in the hot season, when compared with that in the cold season, further they stated that creatinine was not affected by seasons. The values obtained in this study indicated that the elephants were in their healthy status and terrified the overall wellness of those captive elephants.

3.4. Liver enzymes

Liver enzyme monitoring helps to assess the health of the liver as well as the overall health of the animal. AST occurs in higher levels in liver, cardiac and skeletal muscles and is a more reliable indicator for liver diseases. In the present study, the mean value for serum AST was 25.11±1.80 U l^{-1}, which is similar to the value reported by Fowler and Mikota (2006), but it was higher than the mean value (10.2±3.8 U l^{-1}) reported by Sreekumar and Nirmalan (1992) in Indian elephants. Brown and White (1976) reported the AST level in African elephants, which had an average serum value of 19.7 U l^{-1}. Steyrer et al. (2021) reported a mean value of AST as 21±6 U l^{-1} in African elephants. These variations in serum AST levels between the elephant species could possibly be related to their feeding behaviour, drinking habit and metabolic changes between species of elephants. The present study revealed that there was no significant difference among age groups. This was in agreement with Fowler and Mikota (2006) who also observed that AST was not affected by age and gender.

The mean value of ALT level from the investigated elephants of this study was 7.09±0.50 U l^{-1}, which was higher than the ALT level of 1.5–3.0 U l^{-1} reported by Fowler and Mikota (2006). ALT is generally considered not much a useful test for elephants in the diagnosis, since the serum ALT levels were observed to be low in elephants (Fowler and Mikota, 2006). This study findings were also in agreement with Kaneko (1989) and Sreekumar and Nirmalan (1992) who stated that livers of mature horses, cattle, sheep and goats did not contain appreciable levels of ALT and only small elevations occurred from hepatic changes in these species.

Mean value for serum ALP level in this study was 281.02±12.35 U l^{-1} and these values were higher than the mean values (136.2±24.2 U l^{-1}) reported by Fowler and Mikota (2006) and the mean values (68±23 U l^{-1}) reported by Steyrer (2021) in African elephants. Brown and White (1976) reported a serum ALP level of 131.5 U l^{-1} in African elephants, which was lower than mean value observed from this study. The present study also revealed that there were no significant differences between the age groups. However, Fowler and Mikota (2006) reported that the ALP levels were typically higher in young growing mammals, including elephants. Mean value for serum lactate dehydrogenase (LDH) observed in the present study was 352.41±12.03 U l^{-1}, which was similar to the values (366±12.0 U l^{-1}) reported by Sreekumar and Nirmalan (1992).

Gamma-glutamyl transferase is a biliary enzyme and is monitored for assessing the hepato biliary health of animals. The mean value of serum GGT level was 9.78±0.97 U l^{-1} in this study and it was within the range values reported by Fowler and Mikota (2006), a higher mean value (11±2.00 U l^{-1}) was reported by Steyrer et al. (2021) in African elephants. GGT being a liver specific indicator of cholestasis, it is commonly used in large mammals. The variation in GGT levels between the present study and previous studies may be attributed to the variations in age of animals, their physiological conditions etc. Niemuller et al. (1990) reported an increased level of GGT in musth Asian elephant bulls. However, in the present study no major signs suggestive of hepato-biliary involvement was evident and all the elephants were apparently healthy.

3.5. Total bilirubin and direct bilirubin

Liver function is monitored with total and direct bilirubin in all the animals. The mean values of total bilirubin and direct bilirubin in this study were 0.20±0.01 mg dl^{-1} and 0.26±0.03 mgdL^{-1} respectively. These observed values were lower than the findings of Silva and Kuruwita (1993) who reported a total bilirubin value of between 0.23 to 0.94 mg dl^{-1}. This variation might be due to the changes in feed and feed quality across various geographical locations. Elevated

level of total bilirubin (4.94 mg dl^{-1}) was observed in a 30-year-old Asian female elephant with colic resulting from overfeeding (Fowler and Mikota, 2006). Cholesterol value observed in this study was 48.63±2.82 mg dl^{-1} and it was in agreement with the values reported by Fowler and Mikota (2006), however, the mean values described by Silva and Dangolla (2002) were higher than the values observed in this study. The reasons for variations in cholesterol level could be due to the differences in feeding regimen and stable feed availability under captive conditions. The observations of normal value indicated the overall good health status of these captive elephants.

3.6. Muscle enzymes

Muscle enzymes are essential in monitoring the musculo-skeletal heath of animals. A mean serum value of 182.69±12.92 U l^{-1} was observed for serum Creatine kinase (CK) in the studied elephants. It was higher than the values (51.2 ±7.4 U l^{-1}) reported in Indian elephants by Sreekumar and Nirmalan (1992). Steyrer et al. (2021) reported a mean value of CK of 203 U l^{-1} in African elephants which was little lower than the value observed in this study. The variations in enzyme levels observed between the present study and previous studies might possibly due to variations in age of the animals and their physiological conditions during sampling. While LDH is present in all tissues, its activity is highest in muscle and liver, CK is skeletal and cardiac muscle specific. CK elevations occur within a few hours of muscle damage, returning to normal within few hours following cessation of further damage. During muscle injury, elevated creatine kinase helps to determine whether elevated LDH is of muscle or liver origin (Fowler and Mikota, 2006). Lack of any abnormal elevations in this study indicated that the study elephants had a good musculo-skeletal heath.

3.7. *Calcium, phosphorus and magnesium*

Mineral profile indicates the overall metabolic health of the animals as well as the deficiency status if any present. In the present study, the mean serum calcium, phosphorus, magnesium, levels were 11.77±0.25 mg dl^{-1}, 5.31±0.16 mg dl^{-1} and 1.97±0.09 mg dl^{-1} respectively. These values were similar to the values reported by Fowler and Mikota (2006). The serum calcium and phosphorus levels generally remain normal unless affected by metabolic diseases and nutrient deficiencies (Fowler, 1986). There were no significant differences between the age groups in the levels of calcium, phosphorus and magnesium in this study. This was in agreement with Brown and White (1977) who had also reported that there were no differences based on the age and sex of elephants. Further they reported that the diet and environmental factors affected the intestinal absorption and serum levels of both calcium and magnesium.

The values estimated in this study may be used as regional reference range for assessing the health status of captive Asian elephants in Tamil Nadu. However, further studies to evaluate the variations associated with the age, gender and season on serum biochemical parameters, would be of additional help in the health management of captive elephants.

4. CONCLUSION

The regional reference values for the serum biochemical parameters of 46 captive elephants maintained in Tamil Nadu were presented. With no previous large population studies were available in Tamil Nadu, the reference range will help not only in the health monitoring, but also in diagnostic and therapeutic planning in the captive elephants. These valuescan be used by the veterinarians for proper therapeutic management, provided that the feeding pattern, geographic location, immobilization techniques are taken into consideration when interpreting results.

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