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SERUM LEVELS OF SOME BIOCHEMICAL CONSTITUENTS OF CAPTIVE SRI LANKAN ELEPHANTS (*Elephas maximus maximus*)

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ABSTRACT

The serum levels of ten biochemical constituents of Sri Lankan adult captive elephants (*Elephas maximus maximus*) were determined. The mean values ± standard deviations and ranges for these constituents were albumin 20.9 ± 4.9, 10.2 –29.8 g/l, total bilirubin 0.26 ± 0.05, 0.16 - 0.40 mg/dl, creatinine 2.03 ± 0.60, 1.00 – 3.26 mg/dl, urea 15.49 ± 3.89, 7.70 – 22.91 mg/dl, uric acid 0.25 ± 0.09, 0.09 – 0.49 mg/dl, alkaline phosphatase 95.0 ± 30.0, 51.5 – 158.2 U/l, γ glutamyl transferase 3.6 ± 1.8, 1.1 – 7.0 U/l, lactic dehydrogenase 300.7 ± 127.6, 110.1 – 583.4 U/l, glutamic oxaloacetic transaminase 11.7 ± 3.0, 5.7 – 16.8 U/l and glutamic pyruvate transminase 3.7 ± 2.1, 1.0 – 11.4 U/l. There was no statistically significant difference of these parameters between the two sexes. This is the first study to record the serum uric acid, lactic dehydrogenase and glutamic oxaloacetic transaminase levels of Sri Lankan elephants.

Keywords : *Elephas maximus maximus*, Sri Lankan elephant, blood constituents

INTRODUCTION

Scientific data on biochemical constituents of the blood of Sri Lankan elephant (*Elephas maximus maximus*), a sub species of the Asian elephant (*Elephas maximus*) is scarce. The data available includes, serum levels of some electrolytes (Silva & Kuruwita, 1993a; 1993b; Jayasekera & Kuruwita, 1996; Wijesekera *et al.*, 2004), certain hormones (Ratnasooriya *et al.*, 1992a; 1993; Lincoln & Ratnasooriya, 1996; Poole *et al.*, 1997), some enzymes (Silva & Kuruwita, 1993a ; 1993b), glucose (Ratnasooriya *et al.*, 1999), triglycerides (Silva & Kuruwita, 1993a ; 1993b;

Ratnasooriya *et al.*, 2004), total cholesterol (Ratnasooriya *et al.*, 1995 ; 2004), HDL cholesterol, LDL cholesterol, (Ratnasooriya *et al.*, 2004) and total proteins (Silva & Kuruwita, 1993a ; 1993b). However, in view of the critically threatened and endemic status of the Sri Lankan elephant, it is important to collect and document whatever data possible, including those on its serum biochemistry. Such data will not only be useful in the compilation of global information on elephants but also be important in proper diagnosis of diseases and/or disorders, treatment, breeding, general well-being, long term conservation and sound management of both captive and wild elephants of Sri Lanka.

The aim of this study was to investigate serum levels of some selected biochemical constituents, i.e., albumin, total bilirubin, creatinine, urea, uric acid, alkaline phosphatase, γ-glutamyl transferase, lactic dehydrogenase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase of Sri Lankan elephants which are not reported previously. This was done using captive elephants that participated in a cultural pageant, the Colombo Navam Perehara, in February 1997 and 1998.

MATERIALS AND METHODS

A total of 65 (25 males and 40 females), apparently healthy, adult elephants who took part in the Colombo Navam Perehara in February 1997 and 1998 were the subjects of the present study. No attempt was made to differentiate these elephants into five morphotypes.

The blood samples were obtained when the elephant was in a standing position without sedation. About 5-10 ml of blood were collected from a vein or artery on the posterior side of either ear using sterilized butterfly needles (18 gauge) connected to a 20 ml plastic syringe. The entire bleeding period lasted 1.0–1.5 min. All samples were collected at 0900 h – 1130 h taking aseptic precautions.

Blood was allowed to clot at room temperature (28–30 $^{\circ}$ C) and the serum was separated within three hours of collection by centrifugation at 500g for 5 minutes. The serum was stored at –70 $^{\circ}$C until estimation of the biochemical constituents. All the assays were carried out using standard commercially available test kits (Randox, Ireland).

The albumin levels were determined using the Bromocresol purple method as described in the kit. In this method, the serum albumin is bound quantitatively to the indicator 5,5-dibromo-o-cresolsulphonaphthalein (bromocresol purple, BCP) and the absorbance maximum of the albumin-BCP complex is recorded at 600 nm. The

procedure involved mixing the serum sample (10 µl) with the reagent (2.0 ml) containing bromocresol purple, incubating for 2 minutes at 20-25 °C and measuring the absorbance of the sample and standard at 600 nm against a reagent blank. The concentration of albumin in the sample was calculated using the equation, $A_{sample}/A_{standard}$ x concentration of standard. The method is linear up to 60 g/l.

The amount of total bilirubin in the serum was determined using a colorimetric method given in the kit. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by reacting with diazotised sulphanilic acid. In this procedure, the serum sample (0.20 ml) was mixed with sulphanilic acid (0.20 ml), sodium nitrite (0.05 ml) and caffeine (1.00 ml) and allowed to stand for 10 minutes at 20-25 °C. Then, sodium tartrate (1.00 ml) was added and the mixture was incubated at 20-25 °C for 5-30 minutes. The absorbance of the sample was measured at 580 nm against the sample blank. The concentration of total bilirubin (mg/dl) = 10.8 x $A_{sample\ blank}$. The method is linear up to 25 mg/dl.

A colorimetric method described in the kit was used to determine the creatinine levels. Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration. The serum sample (0.1 ml) was mixed with equal volumes of the picric acid and sodium hydroxide solutions provided, allowed to stand for 30 seconds and the absorbance of the sample was measured. Exactly 2 minutes later, the absorbance was measured again. The concentration of creatinine in serum (mg/dl) was calculated using the equation, $\Delta A_{sample}/\Delta A_{standard}$ x 2. The method is linear up to 10 mg/dl.

Urea was estimated using the Urease-Berthelot colorimetric method described in the kit. In this method, urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction. The procedure involved mixing the serum sample (10 µl) with a solution containing sodium nitroprusside and urease (100 µl) and incubating at 37 °C for 10 minutes. Then a phenol solution (2.5 ml) and sodium hypochlorite solution (2.5 ml) were added and the mixture was incubated at 37 °C for 15 minutes. The absorbance of the sample was measured at 550 nm against a reagent blank. The urea concentration (mg/dl) was calculated using the equation, $A_{sample}/A_{standard}$ x 80. The method is linear up to 200 mg/dl.

In order to estimate the uric acid levels, an enzymatic colorimetric method given in the kit was used. In this method, uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3, 5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form

a red-violet quinoneimine compound. The serum sample (20 µl) was mixed with the reagent solution containing 4-aminophenazone, peroxidase and uricase (1000 µl) and incubated for 15 minutes at 20–25 °C. The absorbance of the sample and standard were measured against a reagent blank at 520 nm within 30 minutes. The uric acid concentration was calculated as $A_{sample}/A_{standard}$ x 10. The method is linear up to a concentration of 20 mg/dl.

Alkaline phosphatase (ALP) was determined using the colorimetric method described in the kit. The serum sample (0.01 ml) was mixed at 25 °C with the reagent solution containing p-nitrophenylphosphate. The initial absorbance and the absorbance after 1, 2 and 3 minutes were recorded at 410 nm. To calculate the alkaline phosphatase activity the following formula was used.

ALP (U/l) = 2760 x ΔA 410 nm/min.

The method is linear if the absorbance change per minute does not exceed 0.250.

The γGT levels were determined by the procedure given in the kit by mixing the serum sample (0.10 ml) with the reagent solution containing L-γ–glutamyl-p-nitroanilide and glycylglycine (1.00 ml) at 25°C. The initial absorbance at 410 nm and the absorbance after 1, 2 and 3 minutes were measured. The γGT activity was calculated as 1111 x A 410 nm (U/l). The method is linear if ΔA/ minute does not exceed 0.2 at 410 nm.

The lactate dehydrogenase (LDH) activity was determined using the UV method described in the kit. The serum sample (0.04 ml) was mixed at 25 °C with the reagent solution (1.00 ml) containing pyruvate and NADH. The absorbance was recorded at 340 nm, after 0.5, 1, 2 and 3 minutes. The activity (U/l) was calculated using the formula 4127 x ΔA 340 nm/min. The method is linear provided that the absorbance change per minute does not exceed 0.1 nm at 340 nm.

Glutamic oxaloacetic transaminase (GOT) levels were determined using the UV method given in the kit. The serum sample (0.2 ml) was mixed with the reagent solution containing MDH, LD, NADH, α-oxoglutarate and L-aspartate (1.00 ml) at 25 °C. The absorbance at 340 nm was recorded after 1, 2, 3 and 4 minutes and the activity (U/l) was calculated as 952 x ΔA 340 nm/min. The method is linear if the absorbance change per minute does not exceed 0.16 at 340 nm.

Table 1 : The mean, standard deviation (SD) and range of albumin, total bilirubin, creatinine, urea, uric acid, alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminse (GPT) in the serum of the Sri Lankan elephant (*Elephas maximus maximus*) population sampled.

Serum parameter		Male	Female	Male + Female
Albumin (g/l)	Number	17	26	43
	Mean ± SD	19.4 ± 5.3	21.9 ± 4.5	20.9 ± 4.9
	Range	10.2 – 29.8	14.0 – 28.3	10.2 – 29.8
Total Bilirubin	Number	12	17	29
(mg/dl)	Mean ± SD	0.277 ± 0.067	0.257 ± 0.043	0.266 ± 0.054
	Range	0.160 – 0.400	0.180 – 0.350	0.160 – 0.400
Creatinine (mg/dl)	Number	20	41	61
	Mean ± SD	1.87 ± 0.60	2.10 ± 0.60	2.03 ± 0.60
	Range	1.20 – 3.26	1.00 - 3.17	1.00 – 3.26
Urea (mg/dl)	Number	17	27	44
	Mean ± SD	14.97 ± 4.02	15.81 ± 3.84	15.49 ± 3.89
	Range	8.10 – 22.91	7.70 – 20.84	7.70 – 22.91
Uric acid (mg/dl)	Number	23	29	52
	Mean ± SD	0.27 ± 0.10	0.24 ± 0.08	0.25 ± 0.09
	Range	0.09 – 0.49	0.10 – 0.37	0.09 – 0.49
Alkaline phosphatase	Number	13	26	39
(ALP) (U/l)	Mean ± SD	90.2 ± 31.0	97.0 ± 30.0	95.0 ± 30.0
	Range	51.52 – 158.24	55.2 – 158.24	51.52 – 158.24
γ- glutamyl	Number	22	40	62
transferase (γ–GT)	Mean ± SD	3.7 ± 1.4	3.5 ± 2.0	3.6 ± 1.8
(U/l)	Range	1.4 – 6.7	1.1 – 7.0	1.1 – 7.0
lactic dehydrogenase	Number	25	40	65
(LDH)	Mean ± SD	312.8 ± 132.4	293.4 ± 125.7	300.7 ± 127.6
(U/l)	Range	110.8 – 583.4	110.1 – 582.0	110.1 – 583.4
glutamic oxaloacetic	Number	12	14	26
transaminase (GOT)	Mean ± SD	11.7 ± 3.3	11.7 ± 2.8	11.7 ± 3.0
(U/l)	Range	5.7 – 16.8	8.6 – 16.2	5.7 – 16.8
glutamic pyruvate	Number	16	20	36
transaminase (GPT)	Mean ± SD	3.2 ± 1.6	4.1 ± 2.4	3.7 ± 2.1
(U/l)	Range	1.3 – 7.9	1.0 – 11.4	1.0 – 11.4

Glutamic pyruvate transaminase (GPT) levels were determined using the UV method recommended in the kit. The serum sample (0.2 ml) was mixed with the reagent solution containing LD, NADH, α-oxoglutarate and L-alanine (1.00 ml) at 25 °C. The absorbance at 340 nm was recorded after 1, 2, 3 and 4 minutes and the activity (U/l) was calculated as 952 x ΔA 340 nm/min. The method is linear if the absorbance change per minute does not exceed 0.16 at 340 nm.

Two pre-assayed quality control sera (Randox, Ireland) were used as positive controls to monitor accuracy of the analysis.

The results are represented as means ± SD. Statistical analyses were made using Mann-Whitney U-test (using SPSS statistical software package) to examine whether there is a gender difference of the parameters monitored. Significance was set at $p < 0.05$.

RESULTS

All the serum samples were slightly yellowish in colour without any haemolysis. The results obtained are summarized in Table 1. Of the ten parameters estimated, only total bilirubin, uric acid, γGT and LDH levels were slightly higher in the males. The rest were slightly higher in the females. However, none of the differences was statistically significant ($p > 0.05$).

DISCUSSION

This study recorded ten serum biochemical constituents of Sri Lankan elephants under captive conditions. Blood samples were collected from 65 apparently healthy adult animals which is a sizable number to provide meaningful data. The present number of captive elephants in Sri Lanka is reported to be 186 (Kurt and Mar, 2003). However, it is possible that the numbers are lower than this. Further, in this study sequential blood samplings were not collected due to logistic problems. The serum levels of the biochemical constituents were determined using procedures which are widely used and claimed to be sensitive and reliable. Because of these facts, the data obtained can be considered as representative and be regarded as baseline data for captive elephants in Sri Lanka. It is generally accepted that for each country baseline data on serum biochemical constituents are important.

None of the parameters recorded for Sri Lankan elephant showed a gender difference as reported for serum electrolytes (Wijesekera *et al.*, 2004) or for lipids (Ratnasooriya *et al.*, 2004). However, gender differences are reported for serum glucose (Ratnasooriya *et al.*, 1999) and cholesterol (Ratnasooriya *et al.*, 1995) levels in the Sri Lankan elephants.

Serum levels of GOT, LDH and uric acid were recorded for the first time in the Sri Lankan elephant in this study. Therefore, comparisons with other local studies cannot be made. Both GOT and serum LDH levels were lower than that of Indian elephant (*Elephas maximus indicus*) (Nirmalan & Nair, 1969; Sreekumar & Nirmalan, 1992) and African elephant (*Loxodonta africana*) (Brown & White, 1976; 1980). On the other hand, the serum uric acid level recorded was comparable to that reported for Indian elephant (Lewis, 1994) but was lower than the level reported for African elephant (Brown & White, 1980). These differences could be due to the differences in their major habitats (Indian elephants used were semi-captive / semi-wild and African elephants were wild) or due to species or subspecies differences. Striking differences are reported with certain physiological parameters between the Sri Lankan elephant and the African elephant (eg. in serum chemistry and haematology) (Brown & White, 1976; 1980) and other subspecies of Asian elephants (eg. body temperature, serum cholesterol level, serum glucose level) (Ratnasooriya *et al.*, 1992b; 1995; 1999). Breed differences in serum enzymes are reported in cattle too (Dotta *et al.*, 1964).

γGT, GPT, total bilirubin and alkaline phosphatase levels in the serum of elephants recorded in the present study were lower than the values previously recorded for the Sri Lankan elephants, whilst serum urea was higher (Silva & Kuruwita, 1993a; 1993b). The small sample size of the previous studies (Silva & Kuruwita, 1993a; 1993b) may be a possible reason for this discrepancy. With respect to serum urea however, methodological differences could also be responsible. Interestingly, serum levels of urea and alkaline phosphatase recorded in this study agree well with those reported both for African elephant (Brown & White, 1977; 1980) and Indian elephant (Groneadzka-Ostrowska *et al.*, 1988). However, serum alkaline phosphatase and γGT levels are not reported for Indian and African elephants respectively.

Serum albumin and creatinine levels recorded in this study are similar to those previously reported in Sri Lankan elephants (Silva & Kuruwita, 1993a; 1993b), African elephants (Brown & White, 1980) and Indian elephants (Nirmalan & Nair, 1971; Groneadzka-Ostrowska *et al.*, 1988; Senthilkumar *et al.*, 1999).

Of the ten serum biochemical constituents studied in Sri Lankan captive elephant, serum GOT, LDH and uric acid are recorded for the first time. These values have both academic and clinical significance.

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