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RESEARCH ARTICLE

SERUM BIOCHEMICAL PROFILE OF THE ELEPHANTS IN CHITWAN DISTRICT, NEPAL

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ABSTRACT

Serum biochemical tests are important for routine monitoring of the health status of elephants. The common liver function tests used for elephants include aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase and bilirubin. The research was conducted in 17 elephants of Sauraha at three different locations: Elephant Breeding Centre at Khorsor, Chitwan National Park Hattisar and Gaida Wildlife Camp Hattisar at Sauraha. Various biochemical parameters, i.e. Alkaline Phosphatase, SGOT/AST level, SGPT/ALT level, Blood glucose level and Total Bilirubin level of those elephants were measured using serum samples of those elephants in Motherland Hospital Private Limited, Bhaktapur using standard protocol. In the population of study, blood sugar level was almost in normal range except in one elephant. Serum creatinine level and Total Bilirubin level were almost in normal range. Alkaline phosphatase (ALP) was elevated in four elephants. Among those four elephants, the elephant with slight elevation of Alkaline phosphatase (461 IU/L) had also slight increase in SGPT/ALT value (6 IU/L). However, those with high elevation of ALP had also highly elevated SGPT/ALT value. The SGPT/ALT values of all the elephants were above normal range. The SGOT/AST was almost in normal range except in two elephants. Those with elevated value of SGOT/AST value had also elevated level of ALP and SGPT/ALT. However, the value of Total Bilirubin was almost normal in the sampled elephant populations. Association between SGPT elevation with body condition score was analyzed by using Chi-Square test and elevation of SGPT (above 6 IU/L) was not found to be significantly different ($P = 0.858$, $\chi^2 = 0.032$) between the elephants with low BCS (below 8) and high BCS. Association between SGPT elevation with parasitic presence was analyzed by using Chi-Square test and elevation of SGPT (above 6 IU/L) was not found to be significantly different ($P = 0.585$), $\chi^2 = 0.298$) between the elephants positive for gastrointestinal parasites and negative for gastrointestinal parasites. Association between elevation of alkaline phosphatase with age of elephants was analyzed by using same statistical tool and elevation of alkaline phosphatase was found to be significantly higher ($P < 0.05$, $\chi^2 = 4.65$) in the elephants with age below 6 years compared to higher age group elephants.

INTRODUCTION

Serum biochemical tests are important for routine monitoring of the health status of elephants (Moller *et al.*, 2006; Mikota, 2006 and Vidal *et al.*, 2006). The common liver function tests used for elephants include aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase and bilirubin. Blood gluclose level may be useful to detect various abnormalities like hyperglycemia, stress and diabetes mellitus (Kerr, 2002). The evaluation of biochemical values of blood may provide information that could be helpful for early diagnosis of disease (Mikota, 2006; Thapa, 2010).

Clinical biochemistry involves the basic analysis of samples like blood plasma, urine, feces, CSF and pleural and peritoneal fluids and involves routine tests to determine the health status of animals. Rational selection of various tests helps to determine the abnormalities in animals (Kerr, 2002). Blood glucose, total bilirubin, Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase level might be important to determine the health status of elephants. Elephants have nearly comparable biochemical values as that of equine, bovine, canine and feline species (Silva and Kuruwita, 1993). Some laboratory workers have documented serum biochemical values of the African as well as Asian elephants (Bartles *et all*. 1963; Dillman & Carr, 1970; Brown & White, 1980; Simon, 1961;Sreekumar & Nirmalan, 1989; Sarmah *et al*. 1999 and Wijesekera, Alwis, Vithana & Ratnasooriya, 2008). Normal blood glucose level for elephant is found to be 60-116 mg/dl (Mikota, 2006). In the animal body, there is hormonal control

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over various pathways to maintain a reasonably constant blood glucose level. Glucagon, growth hormone and insulin are required to maintain the blood glucose level in certain range. However, various states are common to cause hyperglycemia and hypoglycemia (Kerr, 2002).

Hyperglycemia can be mild just after meal and it may be extreme in case of uncontrolled diabetes mellitus. Non-diabetic hyperglycemia can be seen in case of stress, exercise and intravenous administration with glucose-containing fluids.

Total Bilirubin level in case of elephants is 0.2 to 1.0 mg/dl (Mikota, 2006). Bilirubin is a by-product of haem breakdown. Increased plasma bilirubin concentrations can be seen in case of fasting hyperbilirubinemia, intravascular hemolysis, liver failure and obstructive biliary disease (Kerr, 2002).

The normal level of SGOT/AST in elephants is 15-35 IU/L (Mikota, 2006). The enzyme is widely distributed in the body particularly in the skeletal muscle, cardiac muscle, liver and erythrocytes. This enzyme is used in most animals to investigate muscle damage and in large animals to investigate liver disease. However, liver disease is not diagnosed solely on the basis of an elevated serum AST/SGOT level (Kerr, 2002).

The normal level of SGPT/ALT in elephants is 1.5-3 IU/L (Mikota, 2006). This enzyme also has similar function to AST. The elevation of this enzyme is related to muscle damage and liver disease as similar to AST/SGOT (Kerr, 2002).

The normal level of ALP in elephants is 60-450 IU/L (Mikota, 2006). This enzyme is also widely distributed in the body particularly in bone, liver and intestinal wall. The higher levels are found in young animals with high osteoblastic activity and lower level is found in liver diseases (Kerr, 2002).

MATERIALS AND METHODS

Study area

The research was conducted in the elephants of Sauraha at three different locations: Elephant Breeding Centre at Khorsor, Chitwan National Park Hattisar and Gaida Wildlife Camp Hattisar at Sauraha.

Fig 1 Map of the research site

Selection of elephants

Altogether 15 elephant calves and 2 adult elephants with calves were selected. Elephant calves belonged to the age group from 4.5 years to 15 years of age while adult elephants were of 43 to 47 years old. Elephants were selected from different locations as given below:

Elephant Breeding Centre (EBC), Khorsor: 10 (8 elephant calves and 2 adult elephants). Chitwan Hattisar of Chitwan National Park (CNP): 5 elephant calves. Gaida Wildlife Camp Hattisar, Sauraha: 2 elephant calves.

Sample collection and processing for biochemical study

From the elephants selected, blood sample (~20 ml) was collected from auricular veins into Serum separator tubes (for blood biochemistry) using 19 gauze winged blood collection sets. Serum was separated within an hour and transferred to cryovials and preserved in deep freeze at -20˚C.

Storage of the sample

The entire samples i.e. whole blood, conjunctival swab, serum and buffy coat were stored at -20˚C until taken to Centre for Molecular Dynamics, Nepal (CMDN), Kathmandu.

Serum biochemistry

Serum samples were submitted to Motherland Hospital Private limited, Bhaktapur for biochemical tests to find the status of biochemical parameters: Alkaline phosphatase, SGOT/AST, SGPT/ALT, Blood sugar level and Total Bilirubin level of each elephants.

Measurement of alkaline phosphatase level

Alkaline phosphatase level in the serum of elephant was measured using “Optimized standard method” (Rick, 1990).
Test kit (Human Gesselschaft für Biochemica und Diagnostica mbh ®) of any size 12217 (16 × 5 ml), 12017 (10 × 10 ml), 12027 (8 × 50 ml) or 12037 (4 × 250 ml) was taken. The test kit composed of Diethanolamine buffer (pH 10.35 ± 2) 1.25 mol/l & Magnesium chloride 0.625 mmol/l as buffer and P-Nitrophenyl phosphate 55 mmol/l as substrate.

Assay

Wavelength	Hg 405 nm (400–420 nm)
Optical path	1 cm
Temperature	25˚C, 30˚C, 37˚C
Measurement	Reading was taken against air (increasing absorbance)

Procedure

The reagent was warmed and the cuvette was fixed to the desired temperature after pipetting the content in fixed amount as below:

Pipette into cuvettes	25˚C, 30˚C, 37˚C
Sample	20 µl
Buffer	1000 µl
Sample and buffer was mixed and was incubated for 1 minute at the desired temperature.	
Substrate	250 µl

The mixture was mixed with substrate and the absorbance was read after 1 minute and at the same time stopwatch was started. The absorbance was read again exactly after 1, 2 and 3 minutes.

From the readings, the mean absorbance change per minute (ΔA/min.) was calculated. Then, the alkaline phosphatase activity in the sample was calculated using the factor: U/L = ΔA/min. × 3433.

Measurement of SGOT/AST level in blood

SGOT/AST level in serum of elephant was determined using "Kinetic method" according to the recommendations of the Expert Panel of the International Federation of Clinical Chemistry (IFCC) (Schumann, 2002).

Test kit (Human Gesselschaft für Biochemica und Diagnostica mbh ®) of any size 12211 (16 × 5 ml), 12011 (10 × 10 ml), 12021 (8 × 50 ml) or 12031 (4 × 250 ml) was taken. It composed of TRIS Buffer (pH 7.8) 100 mol/l, L-aspartate 300 mmol/l, LDH_> 0.9 kU/l & MDH_> 0.6 kU/l as buffer/enzyme reagent and 2-oxoglutarate 60 mmol/l and NADH 0.9 mmol/l as substrate.

Assay

Wavelength	**Hg 365 nm, 340 nm or Hg 334 nm**
Optical path	1 cm
Temperature	25˚C, 30˚C, 37˚C
Measurement	Reading was taken against air (decreasing absorbance)

Procedure

The reagent was warmed and the cuvette was fixed to the desired temperature after pipetting the content in fixed amount as below:

Pipette into cuvettes	**25˚C, 30˚C**	**37˚C**
Sample	200 µl	100 µl
Buffer	1000 µl	1000 µl
Sample and buffer was mixed and was incubated for 5 minute at the desired temperature.		
Substrate	250 µl	250 µl

The mixture was mixed with substrate and the absorbance was read after 1 minute and at the same time stopwatch was started. The absorbance was read again exactly after 1, 2 and 3 minutes.

For ΔA/min within 0.06-0.08 (Hg 365 nm) or 0.12-0.16 (Hg 334 nm, 340 nm), only the measurements from the first 2 minutes was used.

U/l = ΔA/min ×	**25˚C, 30˚C**	**37˚C**
Hg 334 nm	1173	2184
340 nm	1151	2143
Hg 365 nm	2132	3971

Measurement of SGPT/ALT level in blood

SGPT/ALT level in serum of elephant was determined using "Kinetic method" according to the recommendations of the Expert Panel of the International Federation of Clinical Chemistry (IFCC) (Schumann, 2002).

Test kits (Human Gesselschaft für Biochemica und Diagnostica mbh ®) of any size 12212 (16 × 5 ml), 12012 (10 × 10 ml), 12022 (8 × 50 ml) or 12032 (4 × 250 ml) was taken. These composed of TRIS Buffer (pH 7.5) 150 mol/l, L-alanine 750 mmol/l and LDH_> 1.2 kU/l as Buffer/Enzyme reagent and 2–oxoglutarate 90 mmol/l and NADH 0.9 mmol/l as substrate.

Assay

Wavelength	**Hg 365 nm, 340 nm or Hg 334 nm**
Optical path	1 cm
Temperature	25˚C, 30˚C, 37˚C
Measurement	Reading was taken against air (decreasing absorbance)

Procedure

The reagent was warmed and the cuvette was fixed to the desired temperature after pipetting the content in fixed amount as below:

Pipette into cuvettes	**25˚C, 30˚C**	**37˚C**
Sample	200 µl	100 µl
Buffer	1000 µl	1000 µl
Sample and buffer was mixed and was incubated for 5 minute at the desired temperature.		
Substrate	250 µl	250 µl

The mixture was mixed with substrate and the absorbance was read after 1 minute and at the same time stopwatch was started. The absorbance was read again exactly after 1, 2 and 3 minutes.

For ΔA/min within 0.06-0.08 (Hg 365 nm) or 0.12-0.16 (Hg 334 nm, 340 nm), only the measurements from the first 2 minutes was used.

U/l = ΔA/min ×	**25˚C, 30˚C**	**37˚C**
Hg 334 nm	1173	2184
340 nm	1151	2143
Hg 365 nm	2132	3971

Measurement of blood glucose level

Trinder's (1969) recommended method for the determination of blood glucose level from the serum of elephants were followed.

Test kit (Crest Biosystems ®) was taken and it was composed of Glucose reagent (L1) and 100 mg/dl Glucose Standard (S).

Assay

Wavelength	**505 nm (Hg 546 nm) /Green**
Optical path	1 cm
Temperature	37˚C

Procedure

Pipetting was done into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T):

Addition sequence	**B (ml)**	**S (ml)**	**T (ml)**
Glucose reagent (L1)	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose Standard (S)	-	0.01	
Sample	-	-	0.01

After mixing, the composition was incubated at 37˚C for 10 minute or at 25˚C for 30 minute. Then, the absorbance of the standard (Abs. S) and test sample (Abs. T) were measured against the blank within 60 minute.

The Serum glucose level in the sample was calculated using the factor:

$$G\ (mg/dl) = 100 \times \frac{Abs.\ T}{Abs.\ S}$$

Measurement of Total Bilirubin level

The method recommended by Jendrasski and Grof (1938) to quantify the Total Bilirubin level in the blood was used.

Test kit (Crest Biosystems ®) was taken and it was composed of Total Bilirubin reagent (L1), Total Nitrite reagent (L2) and 10 mg/dl Artificial Standard (S).

Assay

Wavelength	546 nm/Yellow-Green
Optical path	1 cm
Temperature	Room temperature

Procedure

Pipetting was done into clean dry test tubes labeled as Blank (B) and Test (T):

Addition sequence	B (ml)	T (ml)
Total Bilirubin reagent (L1)	1.0	1.0
Total Nitrite reagent (L2)	-	0.05
Sample	0.1	0.1

After mixing, the composition was incubated at room temperature for 10 minute. Then, the absorbance of the test sample (Abs. T) was measured against their respective blanks.
Total Bilirubin in mg/dl was calculated as:
B (mg/dl) = Abs. T ×13.

RESULTS

Alkaline phosphatase

Alkaline phosphatase level in sampled elephants ranged from 131 IU/L to 716 IU/L. Four elephants had the value more than normal range (60-450 IU/L) (Figure 2).

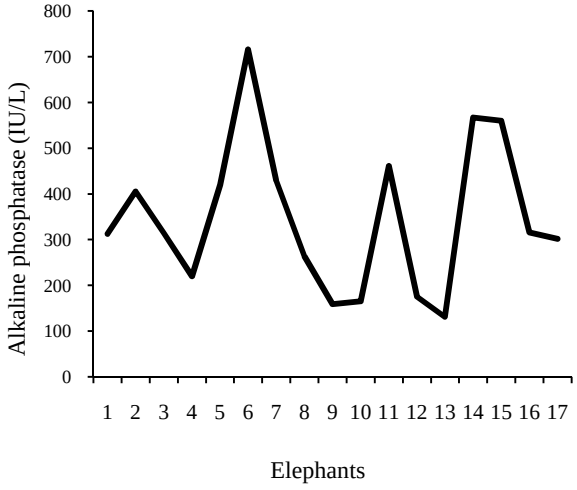

Figure 2 alkaline phosphatase levels in tested elephants

SGOT/AST level

SGOT/AST level in sampled elephants ranged from 5 IU/L to 79 IU/L. one elephant had the value below normal range (15-35 IU/L) and one elephant had the value above normal range (Figure 3).

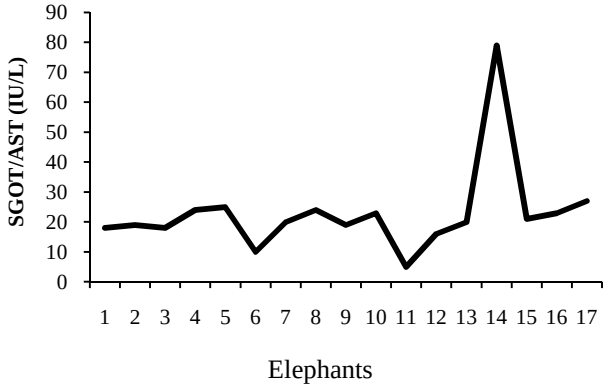

Figure 3 SGOT/AST level in tested elephants

SGPT/ALT level

SGPT/ALT level in sampled elephants ranged from 4 IU/L to 9 IU/L. All the elephants had the value above normal range (1.5 to 3 IU/L) (Figure 4).

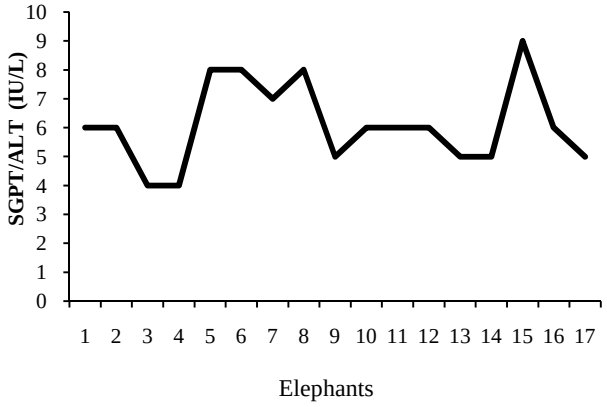

Figure 4 SGPT/ALT level in tested elephants

Blood glucose level

Blood glucose level in sampled elephants ranged from 68 to 884 mg/dl. Three elephants had this value above normal range (60-116 mg/dl). Among those three abnormal values, two had slightly increased values (119 and 131 mg/dl). However, one value was highly increased (Figure 5).

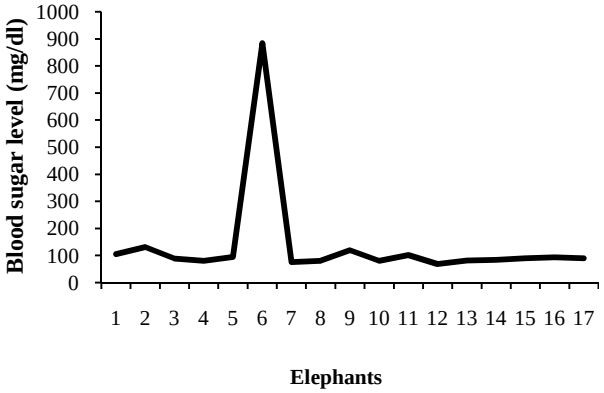

Figure 5 blood sugar levels in tested elephants

Total Bilirubin level

Total Bilirubin level in sampled elephants ranged from 0.48 to 1.12 mg/dl. One elephant had the value above normal range (0.2-1.0 mg/dl). However, the high value was nearly normal (1.12 mg/dl) (Figure 6).

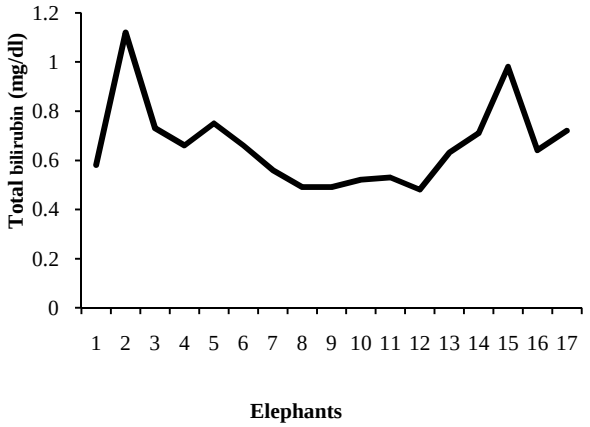

Figure 6 Total bilirubin level in tested elephants

Variation of serum biochemical parameters in the elephants

In the population of study, blood sugar level was almost in normal range except in one elephant. Total Bilirubin level was almost in normal range. Alkaline phosphatase (ALP) was elevated in four elephants. Among those four elephants, the elephant with slight elevation of Alkaline phosphatase (461 IU/L) had also slight increase in SGPT/ALT value (6 IU/L). However, those with high elevation of ALP had also highly elevated SGPT/ALT value. The SGPT/ALT values of all the elephants were above normal range. The SGOT/AST was almost in normal range except in two elephants. Those with elevated value of SGOT/AST value had also elevated level of ALP and SGPT/ALT. However, the value of Total Bilirubin was almost normal in the sampled elephant populations (Table 1).

Table 1 Serum biochemical values of tested elephants

S.N.	Name of the elephant	Alkaline Phosphatase (IU/L)	SGOT/AST (IU/L)	SGPT/ ALT (IU/L)	Blood Sugar level (mg/dl)	Total Bilirubin (mg/dl)
1	Karnalikali	312	18	6 IU/L	105	0.58
2	Narayanikali	405	19	6	131	1.12
3	Lalgaj	314	18	4	88	0.73
4	Himalgaj	220	24	4	80	0.66
5	Nepalgaj	420	25	8	94	0.75
6	Ramugaj	716	10	8	884	0.66
7	Laxmangaj	429	20	7	75	0.56
8	Simsimkali	264	24	8	80	0.49
9	Devikali	159	19	5	119	0.49
10	Koshikali	165	23	6	80	0.52
11	Loktantrakali	461	5	6	101	0.53
12	Parasgaj	175	16	6	68	0.48
13	Rupkali	131	20	5	81	0.63
14	Shovakali	567	79	5	83	0.71
15	Sarasotikali	560	21	9	89	0.98
16	Kushprasad	316	23	6	93	0.64
17	Damdamkali	302	27	5	89	0.72

Relation of serum biochemical profile with body condition score (BCS), parasitic presence and age of elephants

Association between SGPT elevation with body condition score was analyzed by using Chi-Square test and elevation of SGPT (above 6 IU/L) was not found to be significantly different ($P = 0.858$, $\chi^2 = 0.032$) between the elephants with low BCS (below 8) and high BCS. Association between SGPT elevation with parasitic presence was analyzed by using Chi-Square test and elevation of SGPT (above 6 IU/L) was not found to be significantly different ($P = 0.585$), $\chi^2 = 0.298$) between the elephants positive for gastrointestinal parasites and negative for gastrointestinal parasites.

Association between elevation of alkaline phosphatase with age of elephants was analyzed by using same statistical tool and elevation of alkaline phosphatase was found to be significantly higher ($P < 0.05$, $\chi^2 = 4.65$) in the elephants with age below 6 years compared to higher age group elephants.

DISCUSSION

Total Bilirubin level

The value was also found to be in normal range, indicating absence of any hepatic abnormalities and intravascular hemolysis.

Blood sugar level

Blood sugar level was found to be in normal range in 14 elephants, indicating absence of stress, exercise, and non-diabetic hyperglycemia. The two elephants also showed slight elevation (mild hyperglycemia), indicating that those elephants were having meal just before sampling. However, one elephant had extremely high level of blood glucose, the cause of this condition could not be ascertained and need further following studies.

SGOT/AST level

The value was found almost in normal range except in one elephant, indicating some kind of muscle damage or liver disease in that elephant. The elevation of the enzyme was found in the elephant with lower BCS. So, we can consider that the lower BCS might also be due to the muscle damage.

Body Condition Scores (BCS) by BCS only

All the elephants had the value above normal range. The elevation might indicate some kind of muscle damage or liver disease in those elephants. Similarly, the elevation (more than 6 IU/L) was not significantly different ($P > 0.05$) with parasitic prevalence. This fact also suggest for the absence of any liver affection from parasites. Similarly, there is no significant association of the elevation of this enzyme with Body Condition Score (BCS). However, the value of this enzyme was elevated in the elephants with lower BCS, indicating some kind of muscle fatigue and damage in such elephants.

Alkaline phosphatase level

Four elephants had the elevated value of this enzyme. The level of this enzyme was significantly higher in the elephants with age below 6 years. This might be due to higher osteoblastic

activity in the younger age. As this value is not subnormal in any elephant, there were no any hepatic diseases in these elephants.

In short, we found that majority of elephants were of young age as there is elevation of ALP level. Similarly, there is no elevation of Total Bilirubin level and there is no lowering of Alkaline phosphatase, indicating no any hepatic abnormalities. The SGPT/ALT level elevation in all the elephants and SGOT/AST level elevation in some elephants indicated the presence of some kind of muscle damage in the elephant population.

The elevation of blood glucose level in some elephants might be due to stress caused by muscular disease. However, the muscle damage might not be extreme because the elevation of the SGPT/ALT is moderate in the elephant population. Majority of elephants are in the age group, in which training procedures are conducted. This might be the cause for the muscle damage in those elephants.

SUMMARY AND CONCLUSION

During the biochemical analysis, Alkaline Phosphatase was elevated in some elephants indicating high osteoblastic activity in young aged elephants.

The absence of any renal abnormalities might be associated with absence of EEHV infection. Total Bilirubin level was also normal indicating hepatic soundness. Total sugar level was almost normal except in one elephant indicating stress in that elephant. The SGOT/AST level was elevated in one elephant indicating muscle damage in that elephant because it is not associated with elevation of bilirubin level. The SGPT/ALT level was elevated in all the elephants indicating muscle damage in all the elephants.

Elevation of SGPT was not found to be significantly different between the elephants with low BCS and high BCS. Similarly, elevation of SGPT was not found to be significantly different between the elephants positive and negative for gastrointestinal parasites. However, elevation of alkaline phosphatase was found to be significantly higher ($P < 0.05$, $\chi^2 = 4.65$) in the elephants with age below 6 years compared to higher age group elephants

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