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HEMATOLOGICAL PROFILES OF THE MALAYAN SUN BEAR (*HELARCTOS MALAYANUS*) KEPT IN CAPTIVITY

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SUMMARY

Nineteen haematological parameters for the Malayan sun bear which include PCV, RBC count, differential counts for leucocytes, plasma protein, PT and APTT were evaluated. Twenty-six biochemical parameters were analysed namely sodium, potassium, chloride, inorganic phosphate, blood urea nitrogen, creatinine, glucose, cholesterol, total bilirubin, total protein, alanine transferase, alkaline phosphatase, aspartate aminotransferase, craetine kinase, globulin, albumin, globulin-albumin ratio, amylase, lactate dehydrogenase, lipase, lactate, uric acid, triglyceride and gamma-glutamyl transferase. Comparing males and females, males showed a significantly higher value for haemaglobin. Leucocytes and segmented neutrophils were significantly higher in sub-adults. Current values showed low haemaglobin and leucocytes compared to previous data. Preliminary data on anticoagulation factors namely PT and APTT were recorded. Biochemistry showed ALT, GGT and CK were significantly higher in males. The sub-adult group showed higher values of ALT, GGT, CK, LDH and albumin. Compared to previous reports, current data showed lower values of calcium, inorganic phosphate, BUN and AST. Additional preliminary data on GGT, amylase, CK, total triglyceride, lipase and lactate were recorded.

Keywords: Haematology, serum biochemistry, captivity, Malayan sun bear, *Helarctos malayanus*

INTRODUCTION

In order to conserve sun bears from extinction, extensive conservation programmes and research need to be conducted in Malaysia and other Asian countries. In Malaysia, projects such as the Bornean Sun Bear Conservation Centre (BSBCC) have been organised by the Land Empowerment Animals People (LEAP), Sabah Wildlife Department and Sabah Forestry Department (LEAP Malaysia, 2008). The aims are to help rescue and house captive bears, facilitate programmes to increase public awareness and rehabilitate young bears for release back into the wild.

One way to monitor the health status of any animal species is via monitoring the clinical pathology parameters. Blood haematology and serum biochemical analyses could be used to assess health, disease status, nutritional status, habitat quality and stress levels of a wild population (Hanks, 1981). The blood profiles are important for monitoring health of endangered species. Blood variables can also be used to predict survivability in reintroduction and translocation programmes (Mathews *et al.*, 2006). Baseline blood profiles of sun bears are necessary to improve clinical evaluation abilities.

Haematological and biochemical profiles of sun bears have been studied previously (Ramsay, 2003; Kuntze *et al.*, 1988; Stuhrberg, 1988; Bush *et al.*, 1980; Seal *et al.*, 1967) but these have been mainly conducted in the temperate climate. There have been no reports on the blood parameters of the local Malayan sun bears. Therefore, this study is important as it describes baseline blood haematology and serum biochemical values for the Malayan sun bear in captivity in two zoos. The objectives were to establish the baseline values for blood parameters among the local Malayan sun bears to evaluate the differences between gender and age and to compare the differences in blood profiles between previous data and current data.

MATERIALS AND METHODS

Animals

In this study, blood sampling was done on nineteen Malayan sun bears. Eight Malayan sun bears (2 males and 6 females) ranging in age from 4 to 24 years were from Zoo Negara, Kuala Lumpur. They weighed between 47 to 83 kg. Another eleven sun bears (2 males and 9 females) were from Zoo Taiping, Perak. The age of the sun bears was estimated to be from 4 to more than 20 years and weighed from 31 to 87 kg. The animals were divided into 3 age classes based on weight and dental

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morphology. They were classified as juvenile (1 – 5 years old) , sub-adult (6 – 10 years) and adult (more than 10 years) (Beecham and Rohlman, 1994). All the animals were fed with a variety of fruits, bread and honey twice daily. Prior to anaesthesia, all sun bears were fasted for 12 hours.

Blood sampling

Five to 10 ml of blood were drawn either from the cephalic or saphenous vein. The area of blood collection was swabbed with 70% alcohol and blood was drawn using 21 or 23G needle (Terumo® needle, Terumo Corp., Tokyo, Japan) directly into a 5 ml syringe (Terumo® syringe, Laguna, Philippines) or using 21G butterfly catheter (Scalp Vein Set®, Shah Alam, Malaysia). Blood was packed into 4 ml plain tubes (BD Vacutainer® serum, Becton-Dickinson, New Jersey, USA) and into 3 ml ethylenediaminetetracetic tubes (BD Vacutainer® K2 EDTA, Becton-Dickinson, New Jersey, USA). All blood tubes were kept in an ice box before being transported to the laboratory. All samples were transported within 24 hours to the Haematology and Clinical Biochemistry Laboratory, Universiti Putra Malaysia (UPM) for analysis.

Two smears from each blood sample were prepared for differential leucocyte count. Sera were harvested by centrifugation at 1500 rpm (EBA 20®, Hettich Zentrifugen, Buckinghamshire, England) for 5 minutes, then the aliquots were transfered into 1.5 ml microcentrifuge tubes (Eppendorf®, Sigma-Aldrich (M), Subang Jaya, Malaysia) and stored at –20°C (Acson® Vertical Freezer AVF-21, Petaling Jaya, Malaysia) for use later.

Laboratory analysis for haematology

Pack cell volume (PCV) was determined by microhaematocrit technique according to the standard method of NCCLS (2000). Blood was mixed thoroughly by using RM-5® synchronised PVC roller (Finemech®, California, USA) and drawn into a haematocrit tube (NRIS®micro haematocrit tube, Vitrex®, Herlev, Denmark) to about ¾ length. The free end was flame-sealed (Labogaz 206®, Campingaz®, France) and the haematocrit tube was centrifuged at 10,000 rpm for 5 minutes (Haematocrit 20®, Hettich Zentrifugen®, Tuttlingen, Germany). The percentage of PCV was read directly from the micro haematocrit reader (Hawksley® Micro Haematocrit Reader, London, England) and then converted into litre per litre (l/l).

Blood smears were stained with Wright stain (W3000 Sigma®, St. Louis, USA) for differential counts. The percentages of lymphocytes, monocytes, basophils, eosinophils and neutrophils were obtained following examination of stained blood film under oil immersion (Nikon® Eclipse 80i, Tokyo, Japan). The differential blood cell counter (Diffcount® II model 10-112, Modulus Data System, Inc, USA) was used to count the cells. Erythrocytes, haemoglobin (Hb), mean cell volume (MCV), mean corpuscle haematocrit cell (MCHC), thrombocytes and white blood cell (WBC) counts were carried out using the Cell-Dyn® 3700 Automatic Analyser (Vet Package, Abbot Diagnostic®, Berkshire, England).

A refractometer (Atago® T2-NE-Clinical, Atago® Co.Ltd, Tokyo, Japan) was used to determine plasma protein. Prothrombin time (PT) and activated partial thromboplastin time (APTT) coagulation test were done using the Diagnostica Stago Start®4 Coagulation Analyzer (Asnieres-su-Siene, France).

Laboratory analysis for serum biochemistry

For biochemistry analysis, serum was processed in an automated chemistry analyser (HITACHI 902® Automatic Analyser, Tokyo, Japan). The following parameters were analysed: sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphate, blood urea nitrogen (BUN), creatinine, glucose, cholesterol, bilirubun (total and conjugated), total protein (TP), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine kinase (CK), globulin (G), albumin (Alb), amylase, lactate dehydrogenase (LDH), lipase, lactate, uric acid, triglyceride and gamma-glutamyl transferase (GGT). Globulin and albumin-globulin ratios were calculated using the following formula:

Globulin = TP – Alb
A/G ratio = Alb/(TP-Alb)

Data analysis

Statistical analysis was done by computing descriptive statistics for the mean, standard deviation (S.D.) and range. In this study, the two-way ANOVA was used to compare the mean difference between groups that had been split based on two independent variables by using SPSS® version 16.0 (2008), a computer programme for statistical analysis to analyse differences in parameters between gender and age. Significant difference was determined at 95% significant levels ($P<0.05$). The results of this study were also compared to published data (Stuhrberg, 1988; Bush *et al.*, 1980) by using mean ± SD of the present study and previous data based on Dunnet *t*-tests of one group as control (current data) and comparing all other groups against it.

RESULTS

Physical examination of captive sun bears indicated adequate body conditions and no evidence of dehydration. All bears were kept in a captive environment and during the course of this study, all animals were apparently healthy. The values between gender and age were compared (Table 1 and Table 2). This study found

Table 1: ANOVA test on the haematology of captive Malayan sun bears (effect of age and gender)

No.	Parameters	Sex		Age		*P*		
		Males ($N = 4$)	Females ($N = 15$)	Adult ($N = 15$)	Sub-adult ($N = 4$)	Sex	Age	Sex x Age
1.	Erythrocytes (10^{12}/L)	5.64 ± 0.68 (4.89 – 6.40)	5.07 ± 0.52 (4.07 – 5.82)	5.13 ± 0.64 (4.07 – 6.40)	5.43 ± 0.13 (5.28 – 5.59)	0.430	0.976	0.189
2.	Haemoglobin (g/L)	148.0 ± 16.08 (136.0 – 170.0)	130.06 ± 10.64 (110.0 – 147.0)	132.6 ± 15.04 (110 – 170)	138.5 ± 5.68 (135.0 – 147.0)	0.175	0.768	0.080
3.	Packed cell volume (L/L)	0.4 ± 0.03 (0.37 – 0.44)	0.37 ± 0.03 (0.31 – 0.42)	0.37 ± 0.03 (0.31 – 0.44)	0.4 ± 0.02 (0.38 – 0.42)	0.598	0.714	0.082
4.	Mean cell volume (fL)	71.75 ± 3.09 (60.0 – 76.0)	73.13 ± 4.08 (68.0 – 81.0)	72.66 ± 3.97 (68.0 – 81.0)	73.5 ± 3.87 (69.0 – 78.0)	0.564	0.800	0.893
5.	Mean corpuscle hematocrit cell (g/L)	367.25 ± 13.45 (357.0 – 386.0)	351.46 ± 15.07 (324.0 – 381.0)	356.93 ± 15.71 (333.0 – 386.0)	346.75 ± 15.52 (324.0 – 358.0)	0.129	0.264	0.931
6.	Leucocytes (10^9/L)	9.52 ± 1.89 (7.24 – 11.6)	9.77 ± 1.99 (6.20 – 13.0)	9.12 ± 1.69 (6.2 – 12.5)	11.95 ± 0.71 (11.4 – 13.0)	0.708	0.021	0.965
7.	Band neutrophil (10^9/L)	0.16 ± 0.06 (0.09 – 0.22)	0.23 ± 0.10 (0.09 – 0.48)	0.23 ± 0.10 (0.09 – 0.48)	0.18 ± 0.07 (0.12 – 0.26)	0.259	0.479	0.947
8.	Segmented neutrophil (10^9/L)	6.43 ± 1.55 (4.92 – 8.47)	6.05 ± 1.43 (4.22 – 8.97)	5.61 ± 1.04 (4.22 – 7.13)	8.09 ± 0.78 (7.20 – 8.97)	0.635	0.002	0.823
9.	Lymphocytes (10^9/L)	1.65 ± 0.61 (0.80 – 2.21)	2.06 ± 0.77 (0.78 – 3.25)	1.87 ± 0.74 (0.78 – 3.25)	2.38 ± 0.68 (1.62 – 3.25)	0.213	0.509	0.456
10.	Eosinophils (10^9/L)	0.81 ± 0.35 (0.46 – 1.25)	0.94 ± 0.48 (0.13 – 2.00)	0.97 ± 0.41 (0.28 – 2.00)	0.7 ± 0.59 (0.13 – 1.53)	0.561	0.291	0.676
11.	Basophils (10^9/L)	0.0 (0.0)	0.01 ± 0.03 (0.00 – 0.01)	0.01 ± 0.03 (0.0 – 0.1)	0.0 (0.0)	0.716	0.716	0.716
12.	Thrombocytes (10^9/L)	374.0 ± 116.35 (248.0 – 525.0)	429.55 ± 133.25 (67.3 – 630.0)	403.75 ± 136.77 (67.3 – 630.0)	470.75 ± 87.30 (379.0 – 563.0)	0.875	0.203	0.335
13.	Plasma protein (g/L)	79.5 ± 5.97 (74.0 – 88.0)	78.53 ± 5.47 (68.0 – 88.0)	78.0 ± 5.29 (68.0 – 88.0)	81.5 ± 5.74 (74.0 – 88.0)	0.391	0.941	0.049
14.	Prothrombin time (second)	10.32 ± 1.13 (9.2 – 11.9)	10.75 ± 1.91 (8.8 – 16.6)	10.3 ± 0.71 (9.2 – 11.8)	11.8 ± 3.44 (8.80 – 16.60)	0.830	0.170	0.744
15.	APTT (second)	18.75 ± 1.53 (17.10 – 20.70)	19.13 ± 2.40 (14.2 – 23.5)	19.35 ± 1.94 (17.1 – 23.5)	18.02 ± 2.94 (14.2 – 21.2)	0.873	0.644	0.450

Values presented are the means ± standard deviation (S.D.) with the range given in brackets. Values for gender and age were compared by using ANOVA at a significant level of $P<0.05$.
N = sample size; 10^{12}/L = tera per litre; g/L = gram per litre; fL = femto (10^{-15})litre; 10^9/L = giga per litre; sec = time in seconds; APTT = activated partial thromboplastin time.

Table 2: ANOVA test on the serum biochemistry of captive Malayan sun bears (effect of age and gender)

No.	Parameters	Sex		Age		*P*		
		Males ($N = 4$)	Females ($N = 15$)	Adult ($N = 15$)	Sub-adult ($N = 4$)	Sex	Age	Sex x Age
1.	Sodium (mmol/L)	134.57 ± 1.11 (133.6 – 135.9)	135.66 ± 3.02 (132.9 – 145.3)	135.48 ± 3.02 (132.9 – 145.3)	135.25 ± 1.53 (133.8 – 137.1)	0.834	0.804	0.517
2.	Potassium (mmol/L)	4.85 ± 0.33 (4.5 – 5.3)	5.04 ± 0.64 (4.6 – 7.2)	5.02 ± 0.66 (4.5 – 7.2)	4.9 ± 0.14 (4.8 – 5.1)	0.699	0.816	0.938
3.	Chloride (mmol/L)	106.07 ± 4.23 (102.4 –112.0)	104.22 ± 2.4 (100.8 – 111.6)	104.92 ± 3.04 (100.8 – 112.0)	103.47 ± 1.73 (101.6 – 105.0)	0.682	0.164	0.250
4.	Calcium (mmol/L)	2.21 ± 0.15 (2.07 – 2.42)	2.16 ± 0.29 (1.41 – 2.81)	2.13 ± 0.28 (1.41 – 2.81)	2.33 ± 0.11 (2.18 – 2.42)	0.742	0.230	0.776
5.	Inorg. phosphate (mmol/L)	1.49 ± 0.15 (1.30 – 1.63)	1.66 ± 0.14 (1.44 – 1.84)	1.64 ± 0.17 (1.30 – 1.84)	1.56 ± 0.12 (1.43 – 1.71)	0.121	0.485	0.909
6.	Urea (mmol/L)	2.52 ± 1.07 (1.20 – 3.40)	3.66 ± 1.61 (1.1 – 7.1)	3.38 ± 1.67 (1.1 – 7.1)	3.55 ± 1.20 (2.3 – 5.2)	0.452	0.615	0.568
7.	Creatinine (μmol/L)	183.5 ± 26.93 (157.0 – 221.0)	160.0 ± 37.03 (64.0 – 217.0)	166.6 ± 38.81 (64.0 – 221.0)	158.75 ± 24.75 (138.0 – 194.0)	0.550	0.470	0.491
8.	Glucose (mmol/L)	5.62 ± 1.86 (3.8 – 8.2)	5.04 ± 0.73 (3.9 – 6.4)	5.16 ± 1.07 (3.9 – 8.2)	5.15 ± 1.0 (3.8 – 6.2)	0.703	0.170	0.020
9.	Cholesterol (mmol/L)	9.65 ± 1.86 (8.09 – 12.36)	9.13 ± 1.86 (7.1 – 13.39)	9.29 ± 2.04 (7.1 – 13.39)	9.06 ± 0.59 (8.27 – 9.71)	0.781	0.751	0.810
10.	Total bilirubin (μmol/L)	0.12 ± 0.12 (0.0 – 0.30)	1.29 ± 2.46 (0.1 – 7.9)	1.30 ± 2.45 (0.1 – 7.9)	0.07 ± 0.05 (0.0 – 0.1)	0.624	0.595	0.670
11.	Conjugated bilirubin (μmol/L)	0.27 ± 0.35 (0.1 – 0.8)	0.12 ± 0.10 (0.1 – 0.5)	0.17 ± 0.2 (0.1 – 0.8)	0.1 ± 0.0 (0.1 – 0.1)	0.408	0.274	0.408
12.	ALT (U/L)	43.85 ± 14.41 (28.0 – 63.0)	30.7 ± 8.03 (19.6 – 43.8)	30.3 ± 8.17 (19.6 – 43.8)	45.32 ± 11.89 (37.4 – 63.0)	0.003	0.001	0.139
13.	ALP (U/L)	69.5 ± 26.31 (42.0 – 93.0)	59.0 ± 24.45 (0.0 – 93.0)	62.06 ± 25.22 (0.0 – 93.0)	58.0 ± 24.77 (39.0 – 93.0)	0.986	0.360	0.223
14.	GGT (U/L)	38.0 ± 24.85 (23.0 – 75.0)	14.33 ± 5.53 (7.0 – 28.0)	16.13 ± 6.36 (7.0 – 30.0)	31.25 ± 30.34 (8.0 – 75.0)	0.0005	0.0005	0.0005
15.	Amylase (U/L)	117.5 ± 81.31 (60.0 – 175.0)	324.1 ± 154.08 (128.0 – 575.0)	289.9 ± 168.75 (60.0 – 575.0)	288.5 ± 187.38 (156.0 – 421.0)	0.113	0.725	0.0005

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No.	Parameters	Sex		Age		P		
		Males ($N = 4$)	Females ($N = 15$)	Adult ($N = 15$)	Sub-adult ($N = 4$)	Sex	Age	Sex x Age
16.	AST (U/L)	88.7 ± 21.05 (64.4 – 115.8)	72.49 ± 19.08 (45.7 – 111.6)	74.48 ± 18.46 (45.7 – 111.6)	81.22 ± 27. 95(48.5 – 115.8)	0.054	0.214	0.136
17.	CK (U/L)	218.75 ± 177.56 (89.0 – 479.0)	125.73 ± 65.34 (51.0 – 281.0)	124.06 ± 61.26 (51.0 – 281.0)	225.0 ± 179.79 (66.0 – 479.0)	0.001	0.001	0.002
18.	LDH (U/L)	1294.6 ± 277.31 (1032.2 – 1660.7)	1168.24 ± 192.31 (775.6 – 1557.2)	1157.2 ± 199.4 (775.6 – 1557.2)	1336.02 ± 216.98 (1206.8 – 1660.7)	0.096	0.043	0.125
19.	Total protein (g/L)	74.02 ± 1.54 (72.8 – 76.0)	72.1 ± 5.81 (61.1 – 82.4)	71.33 ± 4.95 (61.1 – 78.8)	76.9 ± 4.10 (72.8 – 82.4)	0.803	0.347	0.156
20.	Albumin (g/L)	30.87 ± 4.60 (25.8 – 36.1)	27.90 ± 4.40 (21.7 – 36.2)	26.9 ± 3.43 (21.7 – 33.7)	34.65 ± 1.73 (33.1 – 36.2)	0.280	0.003	0.841
21.	Globulin (g/L)	43.15± 5.86 (36.7 – 48.7)	44.19 ± 6.46 (35.0 – 55.5)	44.43 ± 6.52 (35.0 – 55.5)	42.25 ± 5.22 (36.7 – 49.3)	0.474	0.327	0.339
22.	A:G (ratio)	0.72 ± 0.22 (0.5 – 1.0)	0.63 ± 0.16 (0.4 – 0.9)	0.6 ± 0.15 (0.4 – 0.9)	0.85 ± 0.12 (0.7 – 1.0)	0.241	0.111	0.436
23.	Total triglyceride (mmol/L)	2.37 ± 0.78 (1.37 – 3.23)	2.75 ± 0.56 (1.96 – 3.88)	2.78 ± 0.58 (1.96 – 3.88)	2.23 ± 0.58 (1.37 – 2.61)	0.113	0.046	0.174
24.	Lipase[a] (U/L)	0.0 (0.0)	38.5 ± 13.43 (29.0 – 48.0)	38.5 ± 13.43 (29.0 – 48.0)	0.0 (0.0)	0	0	0
25.	Uric acid (μmol/L)	52.25 ± 4.58 (45.4 – 55.1)	50.82 ± 12.43 (26.9 – 68.6)	48.87 ± 11.18 (26.9 – 68.6)	59.55 ± 6.42 (53.9 – 65.7)	0.796	0.298	0.472
26.	Lactate (mmol/L)	2.97 ± 0.90 (2.2 – 3.9)	3.10 ± 0.86 (2.0 – 5.0)	3.0 ± 0.7 (2.0 – 4.3)	3.35 ± 1.36 (2.0 – 5.0)	0.795	0.280	0.337

Values presented are the means ± standard deviation (S.D.) with the range given in brackets. Values for gender and age were compared by using ANOVA at a significant level of $P<0.05$. *a* = comparison cannot be computed because there are less than two non-empty groups.

N = samples size; mmol/L = millimol (10^{-3}) per litre; μmol/L = micromol (10^{-6}) per litre; U/L = units per litre; g/L = gram per litre; ALT=alanine transferase; ALP=alkaline phosphatase; GGT=gamma glutamyltarnsferase; AST=aspartate aminotransferase; CK=crearine kinase; LDH = lactate dehydrogenase; A: G = albumin : globulin

few statistically significant differences in haematological parameters between gender and age. Differences should be interpreted with caution due to the small sample size including low number of males compared to females and a fewer number of sub-adults compared to adults.

Haematology

There was a significant difference between the values of the present study to that of Stuhrberg (1988)(p=0.0005). There was no significant difference in all parameters between males and females except for the haemoglobin. The haemoglobin values obtained (148.0 g/L ± 16.08) were significantly higher in males compared to females (130.06 g/L ± 10.64) at P=0.015. The haemoglobin values reported by Stuhrberg (1988) in a mixed age and sex of sun bears were also high (150 ± 13.3) as compared to values from the present study (134 ± 13.7).

A comparison between adult and sub-adult sun bears showed that the leucocytes and segmented neutrophil values were slightly higher in the sub-adult group (P = 0.021 and 0.002). The values for leucocytes in the sub-adult was 11.95 ± 0.71 x 10^9/L whereas in adults, they were 9.12 ± 1.69 x 10^9/L. Segmented neutrophil values were higher in sub-adults compared to adults with values of 8.09 ± 0.78 x 10^9/L and 5.61 ± 1.04 x 10^9/L respectively. Slightly lower value of leucocytes (8.06 ± 1.23) were observed in this study as compared to the previous values (9.72 ± 1.92) at P = 0.045 (Table 3). In this study, there were two additional parameters that have not been reported in other studies, namely the prothrombin time (PT) and activated partial thromboplastin time (APTT). The values were recorded as 10.65 ± 1.74 and 19.04 ± 2.19 seconds respectively.

Serum biochemistry

ALT, GGT and CK were significantly higher in males compared to females with the respective values being 43.85 U/L ± 14.41 (P=0.003), 38.0 ± 24.85 U/L (P=0.0005) and 218.75 ± 177.56 UL (P = 0.001). The values of ALT (P = 0.001), GGT (P = 0.0005), CK (P = 0.001), LDH (P = 0.043) and albumin (P = 0.003) were found to be higher in sub-adults as compared to adults.

The difference in values between the present and previous studies (Stuhrberg, 1988; Bush *et al.*, 1980) is shown in Table 4. Calcium and inorganic phosphate values were slightly higher (2.50 ± 0.27 and 1.91 ± 0.29) in the previous study compared to current data (2.17 ± 0.26 and 1.62 ± 0.16) respectively. BUN and AST values were significantly higher in the previous studies (3.42 ± 1.56 and 75.90 ± 20.08) compared to the current study (1.88 ± 0.41 and 46.00 ± 2.74). Total protein was significantly high in previous data (85.47 ± 7.12) compared to that of the current study (72.50 ± 7.38). The values for albumin were higher in previous data (48.95 ± 0.11) as compared to the values of the current study (28.53 ± 4.50).

Additionally, serum biochemical analysis was included in the blood serum biochemical parameters and the values obtained for gamma-glutamil transferase (GGT), amylase, creatine kinase (CK), total triglyceride, lipase and lactate were 19.31 ± 15 (U/L), 289.66 162.76 (U/L), 145.31 ± 100.47 (U/L), 2.67 ± 0.61 (mmol/L), 38.5 ± 13.43 (U/L) and 3.07 ± 0.84 (mmol/L), repectively.

DISCUSSION

The current study found no significant difference in erythrocyte values between age and gender. Compared to the study done by Stuhrberg (1988), the erythrocyte value was significantly higher (7.00 ± 0.82) than that of the current study (5.15 ± 0.60). The values may vary according to the bear population used to collect the data, the environmental variables, genetics and age (Rizzi *et al.*, 2010). In addition, the lower values from this study could be due to the effect of anaesthesia or exercise stress. Kuttner and Weisner (1987) found that animals under anaesthesia have a backflow of erythrocytes to the

Table 3: Comparison of haematological parameters between current values and previous values in captive Malayan sun bears. Values are mean ± S.D.

No	Parameters	[a](n = 19)	[b](n = 9)	[c](n = 2)
1.	Erythrocytes (10^{12}/L)	5.15 ± 0.60*	7.00 ± 0.82*	5.05 ± 0.50
2.	Haemoglobin (g/L)	134 ± 13.7*	150 ± 13.3*	*ND*
3.	Packed cell volume (L/L)	0.37 ± 0.035	0.45 ± 0.14	0.37 ± 0.028
4.	Leucocytes (10^9/L)	8.06 ± 1.23*	9.72 ± 1.92*	*ND*
5.	Band neutrophil (10^9/L)	1.49 ± 1.10	0.22 ± 0.10	0.201
6.	Segmented neutrophil (10^9/L)	6.13 ± 1.42	6.60 ± 1.08	7.70 ± 1.46
7.	Lymphocytes (10^9/L)	1.98 ± 0.75	2.12 ± 0.36	1.92 ± 0.21
8.	Monocytes (10^9/L)	0.48 ± 0.16	0.48 ± 0.33	0.47 ± 0.21

[a]Current values for sun bear, [b]Values reported by Stuhrberg (1988), [c]Values reported by Bush *et al.* (1980). *Mean values having superscripts are significantly different (P < 0.05); 10^{12}/L = tera per litre; g/L = gram per litre; L/L = litre per litre; 10^9/L = g

Table 4: Comparison of serum biochemistry parameters between current values and previous values in captive Malayan sun bears Values are mean ± S.D.

No.	Parameters	[a](n = 19)	[b](n = 9)	[c](n = 2)
1.	Calcium (mmol/L)	2.17 ± 0.26*	2.50 ± 0.27*	*ND*
2.	Inorganic phosphate (mmol/L)	1.62 ± 0.16*	1.91 ± 0.29*	*ND*
3.	Blood Urea Nitrogen (mmol/L)	3.42 ± 1.56*	1.88 ± 0.41*	*ND*
4.	Glucose (mmol/L)	5.14 ± 1.03	*ND*	5.11 ± 3.93
5.	Cholestrol (mmol/L)	9.24 ± 1.83	*ND*	6.60 ± 2.54
6.	Aspartate aminotransferase (U/L)	75.90 ± 20.08*	46.00 ± 2.74*	*ND*
7.	Total Protein (g/L)	72.50 ± 7.38*	85.47 ± 7.12*	*ND*
8.	Albumin (g/L)	28.53 ± 4.50*	48.95 ± 0.11*	*ND*

[a]Current values for sun bear, [b]Values reported by Stuhrberg (1988), [c]Values reported by Bush *et al.* (1980) , *Mean values having superscripts are significantly different ($P < 0.05$); g/L = gram per litre; mmol/L (mmol (10-3/L) per litre; *ND* = No data

spleen, an extravasal fluid shift that effectively dilutes the blood while stress can cause splenic contraction which releases red blood cells into circulation. Haemoglobin was significantly higher in males compared to females. Gender has an effect on red cell mass, as reflected in a research study on beagle dogs (Michaelson *et al.*, 1966) where the males were found to have a slightly higher concentration of haemoglobin than females.

The leucocyte and segmented neutrophil values were slightly higher in the sub-adult compared to the adult groups. Fluctuations of total white blood cell count have been noted in young dogs without any evidence of disease. The study showed that the values remained within the reference interval after maturity (Shifrine *et al.*, 1973). The fluctuation may also be due to the effect of epinephrine or corticosteroid-induced responses during sampling and physiologic stress due to fear or excitement which has been reported to occur in puppies (Meinkoth and Clinkenbeard, 2000; Schultze, 2000). A lower value of leucocytes reflects genetic or environmental factors. Genetic differences between two sub-species of sun bears and raised in different climates may have an effect on blood profiles. Similar differences were observed in healthy North American Belgian Tervuren dogs which exhibited physiologic leukopaenia as compared to Belgian Tervuren dogs in Belgium (Gommeren *et al.*, 2006; Greenfield *et al.*, 2000). Climatic or weather variation may cause minor fluctuation of values.

The PT and APTT are used to measure the time it takes for the plasma to clot (clotting factor). When any of the blood clotting factors are lacking or not functioning properly, the PT and APTT is prolonged (Baker, 2004). Screening tests evaluating the plasma coagulation pathways such as APTT and PT are useful tools in accurately identifying the abnormality and in initiating and monitoring therapy to correct the disorder. Based on the results of our study, PT was considered within the normal range with the mean being 10.65 ± 1.74 (S.D.) compared to the control value (≤ 13.4 seconds) as stated in the manufacturer's guidelines (Diagnostic Stago Stat4). The same results were shown for APTT where the mean of 19.04 ± 2.19 (S.D.) was also within the range of the control results (≤ 34.1 seconds). The results could serve as preliminary data for a sun bear having a clotting disorder.

Variation in calcium and inorganic phosphates was very slight in comparison to previous findings and may be due to the different feeding diet management. The elevated BUN compared to previous data could be due to extra-renal or renal causes and stress, and increased muscular activity due to cortisol (Kaneko, 1997). Significantly elevated AST in this study could be due to stress response due to handling. AST is a non-specific but sensitive marker of soft tissue damage (Kramer and Hoffmann, 1997). A decrease in total protein in this study may be due to the effect of stress (Kaneko, 1997). Total protein was also reflected by the decrease in albumin value; it is known that as the albumin level drops, the total protein value would also decrease. Albumin in this study was significantly low compared to a previous study (Stuhrberg, 1988). Elevated globulin reflects antigenic stimulation due to an unknown pathogen encountered by wild animals (Weber *et al.*, 2002).

Gamma-glutamyl transferase (GGT) levels may be used to determine the cause of an elevated ALP. Both ALP and GGT are elevated in diseases of the bile duct and in some liver diseases. Elevated GGT level indicates a damaged liver but is not specific. The GGT result (19.31 ± 15 U/L) is high compared to that of dogs (3.5 ± 1.8 U/L), but the values are higher in sheep, goats and pigs with values being 33.5 ± 4.3 U/L, 38 ± 13 and 35 ± 21 U/L, repectively (Kaneko *et al.*, 1997). It is not known whether the results are within normal range because there have been no previous reports or data on sun bear or ursids. The results are comparable to other species of domestic animals.

Amylase which is produced in the pancreas and the salivary glands is an enzyme that helps in digestion of

carbohydrates. When the pancreas is not healthy or inflamed, amylase will be released into the blood (Dugdale and Longstreth, 2009). The range of amylase values studied in dogs (Kaneko *et al.*, 1997) is between 185 – 700 U/L. However, the values recorded for sun bears in the current study is 289.66 ± 162.76 U/L. The results are similar to the range for other canines.

Creatine kinase test is used to evaluate neuromuscular diseases in which elevated CK levels indicate muscle damage (Kaneko *et al.*, 1997). The CK level in dogs is 6.25 ± 2.06 U/L, whereas in this study the value was high with the mean being 145.31 ± 100.47. This higher value may be due to a syndrome characterised by damage to skeletal or cardiac muscle following a period of intense physical activity known as capture myopathy (Rose, 2005). It is induced by activities such as chemical or physical restraint, transport or being chased. In this study, a chemical agent administered intramuscularly to immobilise the bear took around 4 to 21 minutes before the animals were fully anaesthetised. During this period, the bears showed signs of excitement and the activity may have increased muscular activity. Intense muscular activity leads to anaerobic glycolysis, elevated lactic acid levels and metabolic acidosis (Spraker, 1980).

Triglycerides is the major lipid in adipose tissue and a major source for fat storage in the body. It is common to measure blood lipids together with cholesterol (Lassen and Fettman, 2004). Lipase test is used, alongside an amylase test, to help diagnose and monitor acute pancreatitis, chronic pancreatitis, and other disorders that involve the pancreas (Dugdale and Longstreth, 2009). The lactate test is done primarily to detect and evaluate the severity of hypoxia and lactic acidosis. Lactate concentrations can increase in any condition that decreases the amount of oxygen available to the body which either increases lactate production, and/or decreases lactate clearance (AACC, 2009; Duncan *et al.*, 2003).

In the present investigation, data were collected from a limited number of animals from two zoological gardens. Restraint of the animals for collection of blood samples from other centres was discouraged in order to avoid unwanted stress to the animals. Fairly normal distribution of data in the present investigation gives an indication that the mean values are representative of the haematobiochemical parameters of the sun bears. More accurate data could be obtained with a greater number of animals. The findings from this study will be useful in monitoring health, nutritional status and diagnosis of disease in sun bears.

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REFERENCES

AACC. (2009). Lactate. American Association of Clinical Chemistry (AACC). *http://www.labtestsonline.org/.*

Baker, D.C. (2004). Diagnosis disorders of hemostasis (Chapter 14). In: Veterinary Hematology and Clincal Chemistry. Thrall, M.A., Baker, D.C., Campbell, T.W., DeNicola, D., Fettman, M.J., Lassen, E.D., Rebar, A., Weiser, G. (Eds). Lippincott William & Wilkins, Maryland. pp.179 – 196.

Beecham, J. J. and Rohlman, J. (1994). A Shadow in the Forest: Idaho's Black Bear. University of Idaho Press, Moscow. pp. 245.

Bush, M., Custer, R.S. and Smith, E.E. (1980). Use of dissociative anesthetics for immobilisation of captive bears: blood gas, haematology and chemistry values. *J. Wildlife Dis.* **16 (4)**: 481 – 489.

Dugdale, D.C. and Longstreth, G.F. (2009). Amylase-blood. In: MedlinePlus. *http://www.nlm.nih.gov/medlineplus.* Updated on 2009.

Duncan, R.J., Prasse, K.W., Latimer, K.S. and Mahaffey, E.A. (2003). Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology. Wiley-Blackwell, New Jersey. pp. 450.

Gommeren, K., Duchateau, L. and Paepe, D. (2006). Investigation of physiologic leukopaenia in Belgian tervuren dogs. *J. Vet. Internal. Med.* **20**: 1340 – 1343.

Greenfield, C.L., Messick, J.B. and Solter, P.F. (2000). Results of hematologic analyses and prevalence of physiologic leukopaenia in Belgian tervuren. *J. Am. Vet. Med. Assoc.* **216**: 866 – 871.

Hanks, J. (1981). Characterisation of population condition. In: Dynamics of Large Mammal Population. Fowler, C.W. and Smith, T.D. (eds). pp.47 – 73.

Kaneko, J.J. (1997). Serum proteins and dysproteinemias. In: Clinical Biochemistry of Domestic Animals (5th edition). Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (eds). Academic Press, San Diego. 117 – 138.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (1997). Clinical Biochemistry of Domestic Animals (5th ed.). Academic Press, San Diego. pp. 932.

Kramer, J.W and Hoffmann, W.E. (1997). Clinical enzymology. In: Clinical Biochemistry of Domestic Animals (5th ed.). Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (eds). Academic Press, San Diego. pp.303 – 325.

Kuntze, A., Hunsdorgg, P. and Kuntze, C. (1988). Additional haematological and biochemical findings recorded from clinically intact and pathologically affected ursine animals (*Thalarctos maritimus, Ursus arctos* and *Helarctos malayanus*). *International Symposium on Diseases of Zoo and Wild Animals* **30**: 339-406.

Kuttner, C. and Wiesner, H. (1987). Changes of blood values in Przewalski horses (*Equus przewalski przewalski*) and zebras (*Equus zebra hartmannae*) during chemical immobilisation. *J. Zoo and Wildlife Med.* **18**: 144 – 147.

Lassen, E.D and Fettman, M.J. (2004) Laboratory evaluation of lipids. In: Veterinary Hematology and Clincal Chemistry. Thrall, M.A., Baker, D.C., Campbell, T.W., DeNicola, D., Fettman, M.J., Lassen, E.D., Rebar, A., Weiser, G. (eds). Lippincott William & Wilkins, Maryland. pp.421 - 430.

LEAP Malaysia (2008). Bornean Sun Bear Conservation Centre. Land Empowerment Animals People (LEAP). Kota Kinabalu, Malaysia. <*www.leapspiral.org*>

Mathews, F., Moro, D., Strachan, R., Gelling, M. and Buller, N. (2006). Health surveillance in wildlife reintroductions. *Bio. Cons.* 131: 338 – 347.

Meinkoth, J.H. and Clinkenbeard, K.D. (2000). Normal hematology of the dog. In: Schalm's Veterinary Hematology (5th ed). Feldman, B.F., Zinkl, J.G. and Jain, N.C. (Eds.). Lippincott William & Wilkins. pp.1057 – 1063.

Michaelson, S..M., Scheer, K. and Gilt, S. (1966). The blood of the normal beagle. *J. Am. Vet. Med. Assoc.* **148**: 532 – 534.

NCCLS (2000). Procedure for Determining Packed Cell Volume by Microhematocrit Method. Approved Standard (3rd ed.). National Committee for Clinical Laboratory Standards. Pennsylvania, USA.

Ramsay, E.C. (2003). Ursidae and Hyaenidae. In: Zoo and Wild Animal Medicine (5th ed.). Fowler, M.E. and Miller, R.E. (Eds.). Saunders, Missouri. pp.523-537.

Rizzi, T.E., Meinkoth, J.H. and Clinkenbeard, K.D. (2010). Normal hematology of the dog (Chapter 104). In: Schalm's Veterinary Hematology (6th ed.). Weiss, D.J. and Wardrop, K.J. (Eds.). Blackwell Publishing, Iowa. pp.799 – 810.

Rose, K (2005). Common Diseases of Urban Wildlife: Common Diseases – Myopathy and Trauma. The Australian Registry of Wildlife Health. pp.1 – 11.

Schultze, A.E. (2000). Interpretation of canine leukocyte responses. In: Schalm's Veterinary Hematology (5th ed.). Feldman, B.F., Zinkl, J.G. and Jain, N.C. (Eds.). Lippincott William & Wilkins. pp.366 – 381.

Shifrine, M., Munn, S.L. and Rosenblatt, L.S. (1973). Hematologic change to 60 days of age in clinically normal beagles. *Lab. Animal Sc.* 23: 894 – 898.

Seal, U.S., Swaim, W.R. and Erickson, A.W. (1967). Hematology of the Ursidae. Comparative Biochemistry and Physiology. Pergamon Press, London, UK. **Part A 22** : 451-460.

Spraker, T.R. (1980). Pathophysiology associated with capture of wild animals. In: The Comparative Pathology of Zoo Animals. Montali, R.J. and Migaki, G. (eds.). Smithsonian Institution Press, Washington. pp. 403 – 415.

Stuhrberg, E. (1988). Blood count and biochemistry of blood serum of polar bear (*Thalarctos maritimus*) and Malayan sun bear (*Helarctos malayanus*). *International Symposium on Diseases of Zoo and Wild Animals*. **30**: 389-398.

Weber, D.K., Danielson, K., Wright, S. and Foley, E. (2002). Hematology and serum biochemistry of Dusky-footed Wood Rat (*Neotoma fuscipes*). *J. Wildlife Dis.* **38**: 576 – 582.