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Research Article

HEMATOLOGY AND PLASMA BIOCHEMISTRY OF GREATER MOUSE-EARED BAT *Myotis myotis* (Borkhausen) (CHIROPTERA: VESPERTILIONIDAE) IN TURKEY

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Abstract: In this study, normal hematologic values and some biochemical parameters were examined in wild-caught greater mouse-eared bat *Myotis myotis* (Borkhausen) captured from Koyunbaba Cave in the Kırklareli province, Turkey. Ten apparently healthy animals (five males and five females) were used to determine the hematology and plasma biochemistry parameters including, leucocyte (white blood cell) counts (WBC), hemoglobin (Hb), hematocrit (HCT), albumin (ALB), total protein (TP), cholesterol (CHO), glucose (GLU), triglycerides (TG), Urea (U), creatine (CREAT), alanine transaminase (ALT) and aspartate amino transferase (AST). No statistically significant differences were found between male and female animals for the analyzed parameters. Plasma total cholesterol concentration was similar with the those of other insectivorous bat species whereas extremely higher than frugivorous species. Also, triglyceride level was higher than the frugivorous bats. The high total cholesterol and triglyceride values in *M. myotis* reflect a protein-rich diet of insectivorous bat species. The values recorded in this study could be helpful in evaluating the physiologic and pathologic status of *M. myotis* in the wild for conservation and management of species.

Özet: Bu çalışmada, Kırklareli ilindeki Koyunbaba Mağarası'ndan yakalanan büyük fare kulaklı yarasa *Myotis myotis* (Borkhausen)'te normal hematolojik değerler ve bazı biyokimyasal parametreler incelenmiştir. Lökosit (beyaz kan hücresi) sayıları (WBC), hemoglobin (Hb), hematokrit (HCT), albümin (ALB), total protein (TP), kolesterol (CHO), glikoz (GLU), trigliserit (TG), Üre (U), kreatin (CREAT), alanin transaminaz (ALT) ve aspartat amino transferazı (AST) içeren hematoloji ve plazma biyokimya parametrelerini belirlemek için görünüşte sağlıklı 10 hayvan (5 erkek ve 5 dişi) kullanılmıştır. Analiz edilen parametreler için erkek ve dişi hayvanlar arasında istatistiksel olarak anlamlı bir fark bulunmamıştır. Plazma total kolesterol konsantrasyonu, diğer böcekçil yarasa türlerininkine benzerken, meyve ile beslenen türlerden oldukça yüksektir. Ayrıca trigliserit seviyesi meyve ile beslenen yarasalardakinden daha yüksektir. *Myotis myotis'* teki yüksek total kolesterol ve trigliserit değerleri, böcekçil yarasa türlerinin protein açısından zengin beslenme şeklini yansıtmaktadır. Bu çalışmada kaydedilen değerler, türün korunması ve yönetimi için doğada *M. myotis'* in fizyolojik ve patolojik durumunun değerlendirilmesinde yardımcı olabilir.

Introduction

Bats (the order Chiroptera) are the only mammalian group capable of powered flight because they have wings. They are unique animals in terms of their flight adaptations and their different dietary preferences including frugivory, insectivory, omnivory, carnivory, nectarivory and sanguinivory (Kunz & Fenton 2003). In addition, bats, composing of over 1,400 described species, constitute the second largest mammal group after rodents, making 20% of all mammals (Wilson & Mittermeier 2019). They play important roles in the ecosystem such as dispersal of seeds, pollination and control of insect populations. The order Chiroptera is classified in two sub-orders, the Microchiroptera (microbats) and the Megachiroptera (megabats). The Microchiroptera includes 16 families, whereas the Megachiroptera is represented by only one family (Pteropodidae). The greater mouse-eared bat *Myotis myotis* (Borkhausen, 1797), belonging to the family Vespertilionidae of microbats is a very common insectivorous species in western Palaearctic region.

Bats have attracted a lot of attention, especially in recent years, since they are carriers of many infections such as rabies, Ebola and SARS. Hematological parameters and

biochemical plasma profile are important indicators to evaluate the physiology and health status of captive and wild animals because they may provide information for diagnosis of diseases. Also, they help to the understanding of physiological responses of animals to the environment in conservation and management of the species. The hematological and biochemical profiles of blood are indicators of the physiological, nutritional and pathological conditions of animals (Bush 1991). Knowledge of the plasma biochemistry and hematological characteristics is an important tool for monitoring population health and might be used as indicators of disease, trauma, nutritional status, and environmental alterations, such as changed habitat quality (McLaughlin *et al.* 2007). Both hematological parameters and plasma chemistry of animals can be influenced by various factors such as gender, age, phase of reproductive cycle, environmental conditions, geographic location, disorders, stress and food availability.

Although several studies on hematological and biochemical parameters of several mammalian species have been reported, the data required to reveal the general health profile and physiological status of the animals are insufficient particularly in the order Chiroptera. In addition, only a few studies have been conducted in Turkey on bat blood cells and hematology (Paksuz *et al.* 2009, Albayrak *et al.* 2016, Albayrak & Sarıçam 2019). In these studies, white blood cell count, hematological values and some blood cell measurements were recorded. However, there has been no biochemical study of the bat species in Turkey. The aim of this study is to define the normal values of some hematological and biochemical parameters in both males and females of *M. myotis*, and to obtain data in order to compare them with animals in different conditions such as environmental variables, infection and hibernation.

Materials and Methods

Study area and sample collection

The bat samples were collected from Koyunbaba Cave in Kırklareli province in Thrace Region/Turkey (41°44'54"N, 27°07'27"E). Five male and five female adults were captured during the day using hand nets. Before blood collection, specimens were weighed and their sex were determined. The males presented weights from 25 to 29 g (27±1.87 g) whereas females weighed 26 to 29 g (27±1.41 g). The study was approved by the Trakya University Animal Experiments Local Ethics Committee (Number: 2017.02.04). With this conditional permission, an application was submitted to the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks. This study was approved (72784983-488.04-101793).

Sample collection and analysis

Following sex determination and weighing of the bat specimens captured, blood samples were collected from wing vein of the animals and transferred to lithium heparin tubes and heparinized capillary tubes. After their blood samples taken, all animals were released back to their natural habitats.

Total White Blood Cell Counts (TWBCs) were obtained manually with a Neubauer hemocytometer. Hemoglobin (Hb) was measured by using Sahli's hemometer. For the estimation of hematocrit, microcapillary tubes were centrifuged at 13,000 rpm for five minutes in microhaematocrit centrifuge. Hematochrit readings were carried out by using a micro-hematocrit reader (NUVE, NF-048 Turkey). 0.75 ml blood samples were taken for each animal by puncturing the wing vein and then put in Eppendorf tubes and heparinized capillary tubes. The Eppendorf tubes were centrifuged at 3,000 rpm (Nüve NF 800 R, Turkey) for 10 min and the plasma was separated.

Using commercial kits (Biomedical Biosystems/Spain), Albumin (ALB) (g/dl), total protein (TP) (g/dl), cholesterol (CHO) (mg/dl), glucose (GLU) (mg/dl) triglycerides (TG) (mg/dl), Urea (U) (mg/dl), creatine (CREAT) (mg/dl), aspartate amino transferase (AST) (U/L), alanine transaminase (ALT) (U/L) and plasma values were determined spectrophotometrically (Microlab, Merck 200, Germany).

Statistical analysis

All data of the measured and obtained were expressed as mean value ± standard deviation (SD). Differences between male and female bats were determined with Student's *t*-test. Differences with $p<0.05$ were considered significant.

Results

All captured bats appeared healthy on physical examination. The average body weight was found as 27 g both in males and females (n = 5 for each) bats. WBC, Hb, and HCT values were 7.84 $x10^9$/L, 16.42 g/dL and 50.20%, respectively. TP, ALB, GLU, CHO, TG, AST, ALT, U and CREAT values were 5.09 g/dL, 2.84 g/dL, 118.70 mg/dL, 127.20 mg/dL, 75 mg/dL, 87.20 U/L, 60.50 U/L and 0.90 mg/dL, respectively. The mean ± SD, minimum and maximum values are given in Table 1. No statistically significant differences in measured hematological and biochemical parameters were found between males and females.

Discussion

Bats are the only group of mammals that can fly and need energy for these abilities (Thomas 1975, Jürgens *et al.* 1981). The metabolic cost of flight for bats is great, and oxygen consumption increase by at least 20 times during sustained flight (Bartholomew *et al.* 1964, Carpenter 1975). It is reported that, during flight, microchiropteran bats increase oxygen supply to body cells by more than 30 times compared to resting levels (Thomas & Suthers 1972). The levels of hemoglobin (16.42 g/dl) and Hct (50.20%) in *M. myotis* were within the range of values reported in previous studies for bat species (Jürgens *et al.* 1981, Arévalo *et al.* 1987, Wołk & Bogdanowicz 1987, Korine *et al.* 1999, Albayrak *et al.* 2016, Bandouchova *et al.* 2020).

Table 1. Hematologic values and plasma biochemistry for wild-caught *M. myotis* (n: number of specimens, Min: minimum value, Max: maximum value, SD: standard deviation).

Parameter (unit)	Male (n = 5)		Female (n = 5)		Mean Adult	
	Mean±SD	Min- Max	Mean+SD	Min- Max		P value
Body weight (g)	27.00±1.87	25-29	27.00±1.41	26-29	27	1.000
WBC (/mm^3)	7.75±1.59	5.92-10.14	7.93±2.68	4.85-11.18	7.84	0.901
Hemoglobin (g/dL)	16.32±1.12	14.9-17.90	16.52±1.48	14.9-18.2	16.42	0.820
Hematocrit (%)	51.00±2.74	48-55	49.40±3.65	45-53	50.20	0.455
Albumin (g/dL)	2.86±0.39	2.4-3.40	2.82±0.26	2.5-3.2	2.84	0.854
Total protein (g/dL)	5.18±0.52	4.5-5.90	5.00±0.27	4.6-5.3	5.09	0.514
Glucose (mg/dL)	118.00±12.63	106-138	119.40±17.44	101-145	118.70	0.888
Cholesterol (mg/dL)	128.00±19.04	96-145	126.40±20.07	101-149	127.20	0.900
Triglycerides (mg/dL)	76.60±29.48	45-121	73.40±37.73	31-131	75	0.885
Aspartate amino transferase (U/L)	87.60±12.99	75-109	86.80±11.17	76-102	87.20	0.919
Alanine transaminase (U/L)	62.80±4.15	57-68	58.20±9.96	45-69	60.5	0.368
Urea (mg/dL)	8.30±0.54	7.6-8.90	8.48±0.30	8.1-8.9	8.3	0.533
Creatine (mg/dL)	0.90±0.24	0.58-1.20	0.90±0.23	0.62-1.2	0.90	0.990

Several studies showed that bats have high mean TWBCs, Hct (around 60%) and hemoglobin (180-240 g l^{-1}) levels when compared with terrestrial mammals (Lewis 1977, Jürgens *et al.* 1981, Arévalo *et al.* 1987, 1992, Wightman *et al.* 1987, Viljoen *et al.* 1997). These physiological adaptations for flight is important to meet the oxygen requirements of powered flight. Also, it is reported that animals with small body size have high hemoglobin and Hct values having benefit in increasing the delivery of oxygen to body tissues (Sealander 1965).

There is no difference between females and males in terms of GLU, and plasma level of GLU in *M. myotis* was within the normal mammalian reference range. Plasma GLU level in *M. myotis* was similar to those reported for other bat species. Studies have shown that insectivorous bats have small body size. On the other hand, while the body size of the bats fed with fruits is larger, others with other feeding habits have an intermediate body size (Meng *et al.* 2016). It is also reported that there are negative inter-species relationships with blood GLU levels in studied bat species, and bats with larger body sizes display lower blood GLU levels.

Although some studies have reported some blood parameters of *M. myotis*, they give very limited data and do not include blood biochemistry (Jürgens *et al.* 1981, Arévalo *et al.* 1987, Paksuz *et al.* 2009). In this study, some biochemical blood parameters were examined and most of the recorded values were similar to those in other insectivorous bat species (Table 2). When compared with the data of insectivorous (Vespertilionidae and Molossidae) and frugivorous (Pteropodidae) species in the literature, it was observed that the results obtained for *M. myotis* were consistent with those of other insectivorous species, but were different from those of frugivorous species. TP and ALB values found in this study were consistent with previous reports for *Rousettus aegyptiacus* (Geoffroy) (Korine *et al.* 1999, Moretti *et al.* 2020), *Pteropus alecto* Temminck (McMichael *et al.* 2015), *P. giganteus* (Brünnich) (McLaughlin *et al.* 2007, Hossain *et al.* 2013), *P. vampyrus* (Linnaeus) (Heard *et al.* 2006), *P. melanotus natalis* Thomas (Hall *et al.* 2014) and *Eidolon helvum* (Kerr) (Selig *et al.* 2016). In this study, plasma total CHO concentration was similar with those of other insectivorous bat species such as *Myotis lucifugus* (LeConte), *Tadarida brasiliensis* (Geoffroy) (Esher *et al.* 1973, Widmaier *et al.* 1996) whereas extremely higher than frugivorous species such as *Rousettus aegyptiacus*, *Eidolon helvum*, *Pteropus giganteus, Pteropus vampyrus* and *Pteropus melanotus natalis* (Korine *et al.* 1999, Heard *et al.* 2006, McLaughlin *et al.* 2007, Hall *et al.* 2014, Selig *et al.* 2016). Also, TG level was higher than the frugivorous bats *R. aegyptiacus* (Korine *et al.* 1999) and *P. alecto* (McMichael *et al.* 2015). These differences may be diet related, with higher total CHO and TG values in *M. myotis* reflecting a protein rich diet of insectivorous bat species. WBC in the present study (7.84) is higher than the previous reports (3.3 and 4.86) (Jurgens *et al.* 1981, Albayrak *et al.* 2016). Intraspecific variations in WBC may be caused by capture and handling, physiological or environmental differences, physiological stress and infections.

In conclusion, in this study, we determined the hematology and plasma chemistry data for wild-caught *M. myotis* from Turkey. Hematological values observed in *M. myotis* are consistent with values obtained for other bat species, reflecting the oxygen requirements of flight. In addition, the higher level of total CHO and TG compared to frugivorous species is probably related to the insectivorous diet of *M. myotis*. Hematological and biochemical parameters are important tools for monitoring and determination of the health status of the species. The results obtained from the present study can be used to evaluate the physiologic and pathologic status of *M. myotis*, and also will be comparable with the results of other bat species.

Table 2. Hematologic values and some plasma biochemistry from previous studies for different bat species. Leucocyte (white blood cell) counts (WBC), Hemoglobin (Hb), hematocrit (HCT), albumin (ALB) (g/dl), total protein (TP) (g/dl), glucose (GLU) (mg/dl), cholesterol (CHO) (mg/dl), triglycerides (TG) (mg/dl), aspartate amino transferase (AST) (U/L), alanine transaminase (ALT) (U/L), Urea (U) (mmol/L), creatine (CREAT) (mg/dl). (Some units are converted to obtain standardized results for comparing).

Literature	Species	ALB (g/dL)	ALT (U/L)	AST (U/L)	CHO (mg/dL)	CREAT (mg/dL)	GLU (mg/dL)	Hb (g/dL)	HCT (%)	TG (mg/dL)	TP (g/dL)	U (mg/dL)	WBC ($x10^9$/L)
	Molossidae												
Widmaier *et al.* (1996)	*Tadarida brasiliensis*	-	-	-	215.00	-	-	-	-	-	-	-	-
	Pteropodidae												
Selig *et al.* (2016)	*Eidolon helvum*	3.85	46.20	50.20	23.50	0.29	114.10	14.89	-	-	7.38	8.50	3.19
McMichael *et al.* (2015)	*Pteropus alecto*	3.61	15.84	76.83	-	-	122.52	16.34	47.00	17.00	6.55	8.16	5.96
McLaughlin *et al.* (2007)	*Pteropus giganteus*	3.70	91.80	45.60	46.40	-	-	-	-	-	7.60	-	-
Hall *et al.* (2014)	*Pteropus melanotus natalis*	4.01	9.94	34.06	16.63	0.52	60.54	-	41.70	-	6.52	5.90	8.39
Heard & Whittier (1997), Heard *et al.* (2006)	*Pteropus vampyrus*	-	13.00	32.00	30.00	1.00	152.00	14.60	44.00	-	7.30	-	12.55
Korine *et al.* (1999)	*Rousettus aegyptiacus*	4.85	-	-	9.17	0.65	176.66	17.23	56.60	28.33	7.91	-	-
Moretti *et al.* (2020)	*Rousettus aegyptiacus*	4.16	46.00	-	-	0.58	157.83	12.40	35.00	-	5.76	7.08	4.20
Desai *et al.* (2012)	*Taphozous nudiventris*	-	-	-	-	-	87.90	13.25	-	-	-	-	-
	Vespertilionidae												
Present study	***Myotis myotis***	**2.84**	**60.50**	**87.20**	**127.20**	**0.90**	**118.70**	**16.42**	**50.20**	**75**	**5.09**	**8.3**	**7.84**
Albayrak *et al.* (2016)	*M. myotis*	-	-	-	-	-	-	14.90	57.00	-	-	-	4.86
	M. blythii	-	-	-	-	-	-	13.09	60.20	-	-	-	4.29
Arevalo *et al.* (1987)	*M. myotis*	-	-	-	-	-	-	15.80	43.30	-	-	-	-
	M. nattereri	-	-	-	-	-	-	20.06	59.78	-	-	-	-
Jurgens *et al.* (1981)	*M. myotis*	-	-	-	-	-	-	18.40	51.00	-	-	-	3.30
Schinnerl *et al.* (2011)	*M. elegans*	-	-	-	-	-	-	-	56.50	-	-	-	2.04
Esher *et al.* (1973)	*M. lucifugus*	-	-	-	265.00	-	-	-	-	-	-	-	-
Rashid *et al.* (2016)	*Pipistrellus pipistrellus*	-	-	-	-	-	-	19.79	21.22	-	-	-	15.64

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Trakya University by the number TUHADYEK 2017.02.04.

Conflict of Interest: The authors have no conflicts of interest to declare.

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