International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 14-04-2023, Accepted: 30-04-2023, Published: 15-05-2023
Volume 8, Issue 5, 2023, Page No. 27-30

Studies on cholesterol level in wild caught females of the Embollonurid Bat, *Taphozous kachhensis* (Dobson) in relation with reproductive cycle

M R Thengare[1], A V Dorlikar[2*]
[1] P.G. Department of Zoology, Yashwantrao Chawhan Mahavidyalaya, Lakhandur, Bhandara, Maharashtra, India
[2] P.G. Department of Zoology, Sevadal Mahila Mahavidyalaya, Nagpur, Maharashtra, India

Abstract
Bats play a key role as pollinators and significantly contribute in controlling insects but very scanty information is available on their basic physiology. The aim of the present investigation was to estimate the significant differences in level of cholesterol in female *Taphozous kachhensis* during various stages of the reproductive cycle. Estimation of cholesterol level was done for twelve months representing all stages of the reproductive cycle. During lactation, quiescence, recrudescence and oestrous mean cholesterol level was found to be 149 ± 2.55 mg/dL, 154 ± 2.76 mg/dL, 155 ± 3.21 mg/dL and 158 ± 3.13 mg/dL respectively. During early pregnancy and mid pregnancy mean cholesterol level was found to be 164.91 ± 1.27 mg/dL and 161.08 ± 3.02 mg/dL respectively. Significant decrease in mean cholesterol level was noted during advanced pregnancy when compared with early and mid pregnancy. Mean cholesterol level was observed in the range of 137-173 mg/dL during the entire reproductive cycle in females. Present investigation revealed the significant differences in the level of cholesterol during the reproductive cycle and thus providing the information regarding basal physiological measurement of bats.

Keywords: bat, *Taphozous kachhensis*, chiroptera, cholesterol

Introduction
Order chiroptera is the second most diverse, abundant group of mammals, which is represented by more than 1421 species grouped in 21 families (Simmons and Cirranello, 2020) [12]. Study of the ecological and physiological characteristics of bats as representative of the numerous and thriving order chiroptera is important. The relatively high life expectancy of some species of bats is of great interest. Information of many unknown aspects of the basic biology and physiology of bats is scanty. Bats are of immense importance to human beings for medical research and public health. However baseline values of hematological profiles of many of the species of the bats are not studied. Physiological changes during the reproductive cycle of the bats is related with the changes in the hematological profile of these bats. The present study revealed useful information on basal values of cholesterol level during various stages of the reproductive cycle for research and conservation of this species.

Material and methods
The present study was conducted on females of the Embollonurid bat, *Taphozous kachhensis*. Identification of the animal was done using standard monograph (Bates and Harrison, 1997) [1]. A mist net of the mesh size (10mm) was used to capture the bats. These were collected from from Ambai Nimbi, 45 kilometers from Bramhapuri (M.S.). After capturing the bats, female bats were separated and were brought to the laboratory. These were weighed on the electronic weighing balance and anesthetized with ether. Blood samples were collected from subclavian and pectoral veins without hurting the animal. Blood samples were collected and was centrifuged to separate the serum. After recovery from the anesthesia, all specimens were released in their natural habitat. Auto analyser was used for quantitative estimation of the serum cholesterol.

CHOD/PAP method was used for the estimation of serum cholesterol. Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. Hydrogen peroxide is formed from free cholesterol due to oxidation which then reacts in the presence of peroxidase enzyme with 4-aminoantipyrine and phenol which result in quinoneimine red dye complex. The intensity of the dye is directly proportional to the concentration of cholesterol present in the serum.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

This kit has
Cholesterol reagent (L1) and Cholesterol standard 200 mg/dL (S)

Protocol for test
Sample: (0.01ml serum + Cholesterol reagent (L1) 1.0 ml

Standard: (0.01ml standard + Cholesterol reagent (L1) 1.0 ml

Blank: (0.01ml Distilled water + Cholesterol reagent (L1) 1.0 ml
Mix well and incubate the solution at 37 °C for 5 min. or at room temperature for 15 minutes. Measure the absorbance of the Standard and test sample against the blank within 60 min at 505 nm.

Calculations
Cholesterol in mg/dL = Absorbance of test / Absorbance of Sample X 200

Statistical analysis

Raw data was analyzed to give mean, standard error and significance using Statistical Package for Social Sciences (SPSS 10.0). All graphs in this study were drawn using Microsoft Excel Software.

Results

Mean cholesterol level observed during different stages of reproductive cycle in female *Taphozous kachhensis* and the P-value corresponding to F-statistic value of one way ANOVA (P = 0.0007) is presented in table 1 and 2 respectively. Histograph showing cholesterol level in female *Taphozous kachhensis* during reproductive cycle is presented in figure 1.

Table 1: One-way ANOVA with post- hoc Tukey HSD showing comparison of cholesterol (mg/dL) in female *Taphozous kachhensis* during reproductive cycle.

Reproductive status	No. of Bats	Cholesterol Range in (mg/dL)	Mean ± S.E	Variance	Standard Deviation
Lactation	18	137 – 170	149.00 ± 2.55[a]	117.52	10.84
Quiescence	12	140 – 170	154.00 ± 2.76[a]	91.63	9.57
Recrudescent	06	145 – 165	155.00 ± 3.21[a]	62.00	7.87
Oestrous	06	148 – 168	158.00 ± 3.13[a]	58.80	7.66
Early Pregnancy	12	158 – 170	164.91 ± 1.27[ab]	19.53	4.42
Mid Pregnancy	12	145 – 173	161.08 ± 3.02[ab]	109.53	10.46
Advanced Pregnancy	06	145 – 165	155.00 ± 3.02[a]	54.80	7.40
Pooled Total	72		156.25 ± 1.21	106.04	10.29

Table 2: One-way ANOVA of seven independent groups showing P-value corresponding to F- statistic.

Source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
Treatment	2,225.6667	6	370.9444	4.5460	0.0007
Error	5,303.8333	65	81.5974		
Total	7,529.5000	71			

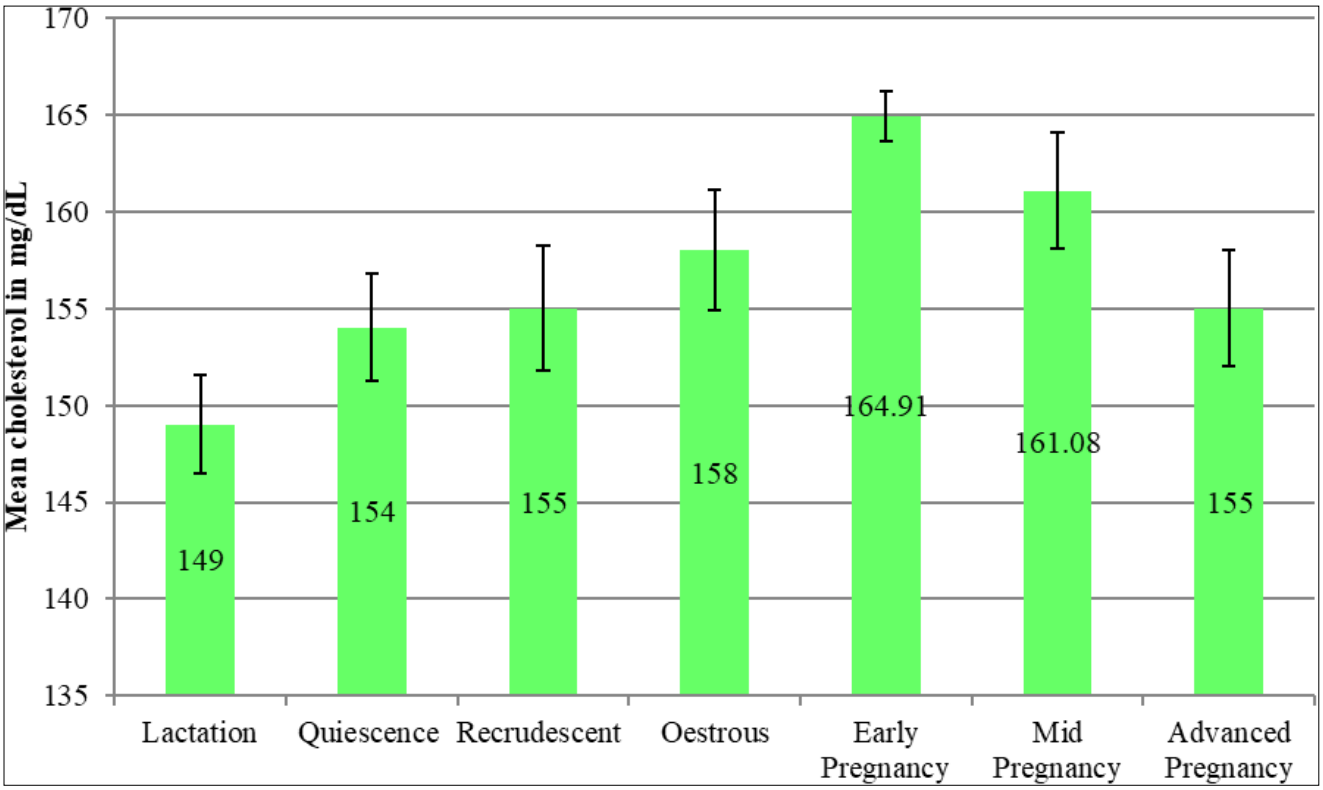

Fig 1: Mean cholesterol in female *Taphozous kachhensis* during reproductive cycle

During lactation, quiescence, recrudescence and oestrous mean cholesterol level was found to be 149 ± 2.55 mg/dL, 154 ± 2.76 mg/dL, 155 ± 3.21 mg/dL and 158 ± 3.13 mg/dL respectively. No significant differences were observed in mean cholesterol level during lactation, quiescence, recrudescence and oestrous stages. Significant increase in mean cholesterol level was noted during early pregnancy and mid pregnancy. During early pregnancy and mid pregnancy mean cholesterol level was found to be 164.91 ± 1.27 mg/dL and 161.08 ± 3.02 mg/dL respectively. Significant decrease in mean cholesterol was observed during advanced pregnancy when compared with early pregnancy and mid pregnancy. Pooled total mean cholesterol level during all stages of reproductive cycle in females was found to be 156.25 ± 1.21 mg/dL. During the entire reproductive cycle in females, mean cholesterol level was observed in the range of 137 – 173 mg/dL.

Discussion

Cholesterol is a lipid which is widely distributed in various types of animal tissues. It is synthesized in the liver and is a normal component of bile and principal constituent of gallstones. Cholesterol acts as a precursor for the synthesis of various steroid hormones like adrenal corticoids and sex steroid in mammals. Total level of cholesterol is related to liver function. Increased level of cholesterol is generally found in pathological conditions like hypothyroidism, nephrosis, coronary artery disease, hyperlipoproteinemias, diabetes mellitus and many liver disorders. Low level of cholesterol occurs during malnutrition, acute infections, haemolytic jaundice, pernicious anemia and hyperthyroidism. In humans normal level of total cholesterol is between 152 to 240 mg/dL. Ageing is associated with an increase in serum total cholesterol. In the present investigation pooled mean total cholesterol in females of *Taphozous kachhensis* found to be 156.25 ± 1.21 mg/dL. In females of *Taphozous kachhensis* no significant variation in total cholesterol were observed during lactation, quiescence, recrudescence, oestrous and advanced pregnancy. Significant elevated level of total cholesterol was noted during early pregnancy and mid pregnancy is associated with higher anabolic activity of the liver for the synthesis of sex steroids like 17-β oestradiol and progesterone. Higher levels of these hormones are required for the maintenance of pregnancy. McMichael et al. (2015) [7] had noted the total cholesterol in wild black flying foxes, *Pteropus alecto* and reported significant differences in the level of cholesterol in females at $P < 0.001$ level. They had noted the plasma total cholesterol females of *Pteropus alecto* was 17.40 mg/dL. Selig et al. (2016) [11] observed mean cholesterol level 23.5 mg/dL in straw colored fruit bats (*Eidolon helvum*). Low level of total cholesterol in pteropodid bats as compared to insectivorous bat are likely due to a low-protein diet, as cholesterol is obtained either by diet or by synthesis within liver (Widmaier et al., 1996; Heard and Whittier, 1997) [13, 4]. Moretti et al. (2021) [8] had observed very low value of triglycerides in healthy captive Egyptian fruit bat *Rousettus agyptiacus*. Esher et al. (1973) [3] had noted 87% decrease in liver cholesterol during torpid condition in *Myotis lucifugus*. Widmaier et al. (1996) [13 had noted the high fasting plasma cholesterol level (215 ± 8 mg/dL) in insectivorous Mexican free tailed bat, *Tadarida brasiliensis mexicana* during late pregnancy and lactation. They had correlated extra ordinary high levels of cholesterol with consumption of double the amount of insect diet which is high in fat.

Normal level of total cholesterol in captive *Pteropus hypomelanus* was 12 ± 4 mg/dL. Such low levels of total cholesterol was related to primary frugivorous habit associated with consumption of fruit (Widmaier et al., 1996; Heard and Whittier, 1997) [13, 4]

Heard and Whittier (1997) [4] had observed the plasma cholesterol level in *Pteropus vampyrus*, *Pteropus rodricensis* and *Pteropus hypomelanus* was 30 ± 14, 33 ± 40 and 17 ± 10 mg/dL respectively. They had observed the cholesterol level in *Pteropus rodricensis* was in the range of 2 to 152 mg/dL. McLaughlin et al. (2007) [6] has found cholesterol level 46.4 ± 0.7 mg/dL in wild caught flying fox, *Pteropus giganteus*. Highest range level cholesterol of *Pteropus rodricensis* shows similarity with our study. Sarmin et al. (2020) noted the cholesterol level in juvenile buckes of *Ettawa* crossbred goats in the range of 68-162 mg/dL. The high cholesterol level in this study was comparable to our finding.

Korine et al. (1999) [5] had observed the seasonal variations in the cholesterol level in fruit eating bat, *Rousettus aegyptiacus*. They have noted the total cholesterol level during winter, spring, summer and autumn were 1.00 ± 0.02, 1.00 ± 0.00, 9.17 ± 2.63 and 2.00 ± 1.41 mg/dL respectively and suggested cholesterol as a function of diet (Carroll and Kurowska, 1995; Widmaier et al., 1996; Heard and Whittier, 1997) [2, 13, 4].

Otis et al. (2011) [9] had studied the cholesterol and lipoprotein dynamics in hibernating squirrels and found similar concentrations of cholesterol during spring and summer. Cholesterol transported by lipoprotein particles in circulation. Excess cholesterol is excreted from the body in the form of bile acid by fecal excretion. They had observed a high level of cholesterol in plasma during hibernation was due to thirteen fold lower expression of cholesterol alpha-hydroxylase enzyme. Low concentration of cholesterol during winter is related to efficient use of lipoprotein in mammals essential for their survival.

References

1. Bates PJJ, Harrison DL. Bats of the Indian Subcontinent. Harrison Zoological Museum Publications, 1997, 258.
2. Carroll KK, Kurowska EA. Soy consumption and cholesterol reduction: review of animal and human studies. J. Nutr.,1995:125:594–597.
3. Esher RJ, Fleischman AI, Lenz PH. Blood and liver lipids in torpid and aroused little brown bats, *Myotis lucifugus.* Comparative Biochemistry and Physiology Part A: Physiology,1973:45(4):933–938.
4. Heard DJ, Whittier DA. Hematologic and plasma biochemical reference values for three flying fox species (*Pteropus* sp.). Journal of Zoo and Wildlife Medicine,1997:28(4):464-470.
5. Korine C, Zinder O, Arad Z. Diurnal and seasonal changes in blood composition of the free living Egyptian fruit bat (*Rousettus aegyptiacus*). Journal of Comparative Physiology B,1999:169:280-286.
6. McLaughlin AB, Epstein JH, Prakash V, Smith C. S., Daszak, P., Field, H. E. and Cunningham, A. A. Plasma biochemistry and hematologic values for wild-caught flying foxes (*Pteropus giganteus*) in India. Journal of Zoo and Wildlife Medicine,2007:38(3):446-452.
7. McMichael L, Edson D, McLaughlin A, Mayer D, Kopp S, Meers J, Field H. Haematology and Plasma Biochemistry of Wild Black Flying-Foxes, (*Pteropus alecto*) in Queensland, Australia. PLoS ONE,2015:10(5):1-13.
8. Moretti P, Ravasio G, Magnone W, Di Cesare F, Paltrinieri S, Pecile A, et al. Haematological, serum biochemical and electrophoretic data on healthy captive Egyptian fruit bats (*Rousettus aegyptiacus*). Laboratory Animals,2021:55(2):158-169.
9. Otis JP, Sahoo D, Drover VA, Yen CLE, Carey HV. Cholesterol and Lipoprotein Dynamics in a Hibernating Mammal. *PLoS ONE.*,2011:6(12):e29111.
10. Sarmin Widiyono I, Anggraeni D. Comparison of Hematological and Biochemical Parameters of Clinically Healthy Buck Kid of Saanen, Sapera, and Ettawa Crossbred in Indonesia International

Symposium on Water, Ecology and Environment. IOP Conf. Series: Earth and Environmental Science,2021:690:012029.
11. Selig M, Lewandowski A, Kent MS. Establishment of reference intervals for hematology and biochemistry analytes in a captive colony of straw-colored fruit bats (*Eidolon helvum*). Journal of Zoo and Wildlife Medicine,2016:47(1):106-112.
12. Simmons NB, Cirranello AL. Bat species of the world: a taxonomic and geographic database. Accessed on, 2020, 7(10).
13. Widmaier EP, Gornstein ER, Hennessey JL, Bloss JM, Greenberg JA, Kunz TH. High plasma cholesterol, but low triglycerides and plaque-free arteries in Mexican free-tailed bats. American Journal of Physiology,1996:271(5-2):R1101-R1106.