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STUDY OF CORRELATIVE CHANGES IN SERUM CREATININE PHOSPHOKINASE AND TESTOSTERONE CONCENTRATION DURING BREEDING SEASON OF MALE FRUIT BAT *ROUSETTUS LESCHENAULTI* (DESMEREST)

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ABSTRACT:
Little is known about the reproductive endocrinology of the male fruit bat *Rousettus leschenaulti* except that adult males show double peaks in their testicular activity corresponding to the two pregnancy cycles of the female first peak occurs during February – March and second occurs during October-November. Studies show that there is association between serum sex homone levels and liver, muscle or heart functions. Creatine PhosphoKinase (CPK) is also a potent marker of heart and liver function. The main aim of this study was to determine the correlation of serum testosterone (T) level and creatinne phosphokinase concentration in male fruit bat *R. leschenaulti*.The present study demonstrate a negative relationship between CPK concentration and circulating testosterone concentration. Data from present study provides important baseline and endocrine information that can be used for the wildlife reproduction and thereof wildlife protection studies on the field.
Key words: - Fruit bat, Testosterone, Creatinine phosphokinase.

INTRODUCTION:

Although Chiropterans are the second largest order of mammals, detailed reproductive patterns and their associated endocrine characteristics have been documented only in a few species (Krutzsch and Crichton, 1990; Bernard et al., 1991; Singh and Krishna, 2000). Sex steroid hormones influence the function of various organs including muscular system, digestive system and heart (Muthusamy et.al., 2011).Testosterone is a hormone from the androgen group primarily secreted in the testicles of males .Studies show that there is association between testosterone level and function of tissues of various typers (Bain J,2007).Creatine kinase (CK) is an enzyme expressed by various tissues. Serum levels of CPK are abnormally changed in kidney, heart and liver or other disorders (Pan et al., 2013; Wallimann et. al., 1992). Kidney and liver damage have been also observed when serum testosterone level is higher than normal, indicating the effects of testosterone on liver and kidney function (Filova et al,.2013;Handelsman and Gupta, 1997).

MATERIALS AND METHODS:

Breeding Habits

The Indian fruit bat, *Rousettus leschenaulti* shows a peculiar breeding cycle. Adult males show double peaks in their testicular weight corresponding to the two pregnancy cycles of the female. The first peak occurs during October-November and the second during February-March (Gopalakrishna and Choudhary, 1977).

Collection of animals

The specimens of *Rousettus leschenaulti* were collected with the help of mist net placed at the entrance of an underground mine of Mansar / Kandri near Nagpur, Maharashtra (20°92"N 78°95"E). Time of collection, body mass, wing

span, length of forearm and other salient features of each specimen were maintained in the field diary. The size of testes were estimated by palpating the longest axis of the testis and measuring this distance with callipers, similarly the width was also measured and then each male was transferred to an individual comfortable cage. These traps were transported to the RTM Nagpur University Laboratory. Minimum noise, human exposure and handling were employed to minimize capture stress and excitement. For each sampling, three bats were used.

Blood sampling

The bats were held in hands and no anesthesia was used at the time of sample collection. 2ml of blood was collected into sterile tube with no anticoagulants (neither EDTA nor heparin) after puncturing a wing vein. After blood sampling each bat was released. Serum creatinine phophokinase(CPK) estimation was performed using a Technicon semiautoanalyzer (RA-50 Technicon, Germany), (IFCC Method) and the measurement of Serum Testosterone was done by Enzyme Linked Immunosorbent Assay (ELISA). For the determination of testosterone level in blood, 2ml. of blood was allowed to clot at room temperature for half an hour. The clotted blood was then used for measurement of serum testosterone by ELISA (Widsdom, 1976).The data was analyzed statistically, standard errors were calculated, on the basis of which graphs were plotted to establish a relationship if any.

RESULT & DISCUSSION:

For the present study evaluation of above mentioned two parameters were performed throughout the annual cycle, the observed data is tabulated in table-1 with related bar diagram. (Fig-1 & 2).

The body weight, testis size, serum testosterone levels and CPK concentrations in the male *Rousettus leschenaulti* collected from January 2007 to December 2007 covering both the breeding periods are depicted in the Table – 1 and bar diagram (Fig.1) have been drawn to show the correlation.

This bat shows a peculiar breeding cycle. Adult males show double peaks in their testicular activity corresponding to the two pregnancy cycles of the female. The first peak occurs during February-March and the second during October-November (Gopalakrishna and Choudhary, 1977).

High serum testosterone concentrations observed during the breeding period (range 18.37-14.60 ng/ml) are coincident with the peak of the mating during February-March (peak-1) and October-November (peak-2) whereas the rapid decrease in serum testosterone concentrations (range 2.73-6.43 ng/ml) during the months May, June, July and August appears to be associated with the end of breeding season. The short peaks during the months September, December, January (range 9.67–10.27 ng/ml) are consistent with the recrudescence period. The higher values of testosterone during breeding season strongly support the notion that hyperandrogenism may be, in part because of enhanced testicular responsiveness to gonadotropin signaling.On the contrary serum level of CPK was found elevated throught the year (957.3 to 998.3 U/L) except in breeding season (900.3 & 900.6 U/L), which are peculiar in sense of their nocturnal habits, extreme sensitivity to light and heat beside capture stress, hyperactivity, physical injuries because of fighting among males. The CPK cocentration was found lowered in the breeding season may be due to increased concentration of serum testosterone which may result in cellular changes in various target tissues leading to decreased serum level of CPK . In accordace with this study some other studies have shown that serum CPK values are significatly decreased after administration of steroids (Hinderks & Frolich, 1979) . Low serum CPK values have also been repeated in conraceptives steroid users (Gupta, 1981). However in contrast to present findings

there are studies showing that chronic administration of testosterone is associated with increased CPK level (Häkkinen & Alén, 1989).

CONCLUSION:

In conclusion the serum Creatinine phosphokinase concentration appears to be correlative to the testosterone concentrations which parallel the known morphological transitions of the testes and body mass. Our study also confirms the presence of seasonal variations in serum CPK concentration and testosterone and its associated changes in the male. These findings contribute significantly to the wild life reproduction and thereof wild life protection and behavioral ecology studies in the field. Our findings also confirm that the male adult *Rousettus* under natural conditions exhibits characteristics of an annual reproductive cycle.

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Specimen (n = 3)	Date of collection	Body weight(g)	Size of Testis (cm)	Testosterone (ng/ml)	Creatinine Phosphokinase (U/L)	Reproductive status
Male	8/1/07	111.33 ± 1.86	2.47 ± 0.03	10.27 ± 0.05	971.00 ± 1.000	Active male
Male	6/2/07	113.33 ± 1.67	2.60 ± 0.06	16.50 ± 0.04	900.67 ± 0.120	Active male
Male	9/3/07	128.00 ± 1.53	2.90 ± 0.10	18.70 ± 0.04	900.37 ± 0.186	Male active spermatogenesis + Leydig cells active Mating period (Peak-1)
Male	8/4/07	100.00 ± 2.89	2.17 ± 0.17	9.70 ± 0.12	961.67 ± 0.882	Active male
Male	8/5/07	98.00 ± 0.58	2.03 ± 0.09	5.73 ± 0.15	994.00 ± 2.309	Male inactive (Quiescence)
Male	9/6/07	95.00 ± 2.65	1.96 ± 0.07	3.13 ± 0.19	998.33 ± 1.667	Quiescent male
Male	9/7/07	93.00 ± 2.52	1.83 ± 0.04	2.87 ± 0.09	986.00 ± 3.512	Quiescent male
Male	6/8/07	70.67 ± 0.67	1.20 ± 0.15	2.73 ± 0.10	978.67 ± 2.963	Male inactive (Quiescence)
Male	4/9/07	71.00 ± 1.0	1.03 ± 0.03	6.23 ± 0.15	992.00 ± 1.155	Recrudescent male
Male	6/10/07	100.67 ± 5.21	2.13 ± 0.19	14.60 ± 0.08	992.33 ± 1.202	Active male
Male	9/11/07	121.67 ± 1.67	3.07 ± 0.07	18.37 ± 0.15	900.37 ± 0.186	Active male showing complete spermatogenesis mating period (peak-2)
Male	11/12/07	99.00 ± 2.08	2.33 ± 0.17	8.73 ± 0.07	957.33 ± 1.453	Active male

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