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Haemocytobiochemical and morphometrical profile of three striped Indian palm squirrel *Funambulus palmarum* (Linnaeus, 1766) with respective to sex

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Abstract

The objective of this study was to investigate and establish the standard reference value of haematological parameters in squirrels. Blood was aseptically drawn from five individuals of each sex and was taken for haematological analyses. The mean values of haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin, and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated. Along with this, the serum biochemical parameters like total protein, albumin, globulin, glucose, and cholesterol were found out and each parameter was expressed as mean±SE of both the sexes of animal. The differential leucocyte number of each category of cell (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) was enumerated. In morphometrical analyses, the cell length and breadth along with the nucleus of erythrocytes, lymphocytes, monocytes, neutrophils, basophils, and eosinophils were also measured. Considering both haematobiochemical and cytomorphometrical aspects, various angles are interpreted.

Keywords: *Funambulus palmarum*, Blood, Haematological analyses, Biochemical analyses, Cytomorphometrical analyses.

Introduction

Indian palm squirrel is a highly agile creature in terrestrial ecosystem which are observed on land and trees. Out of five species of the animal, the very commonly available Indian palm squirrel having three stripes called three striped palm squirrel bearing the Latin name *Funambulus palamarum*, is a mammal of the order Rodentia under the family Sciuridae which is abundant in the states like Tamil Nadu, Odisha and Sri Lanka. Indian palm squirrels are also known as the three striped squirrel due to the presence of dark and white stripes alternately on their backs which has importance in mythology with respect to Ramachandra and the Ramayana. These are found nesting on the tree tops of available tall and big trees. Haematology is the study of blood forming organs which answers and indicates some blood related disorders of the animals[1]. The normal blood composition of a healthy animal including human being reveals the normal healthy activities and performance of the animal. Blood is an essential fluid connective tissue, which is responsible, accountable and answerable for homeostasis[2]. Haematological parameters are the key indicators of the physiological and biochemical status of the animals. In global sphere, haematological investigation and experimentation are significant diagnostics and management protocol in animal and veterinary science to evaluate and asses the health condition of the animals. The haematological profile of an animal provides an opportunity to investigate the metabolic properties of the body[3]. Haemocytobiochemical analysis facilitates to differentiate the normal state from that of stress, which can be nutritional, environmental or physical[4]. Blood and its constituents enable in providing information for the diagnosis and prognosis of diseases. The physiological condition of animals mostly depends upon the configuration and structural orientation of the cell and nucleus along with the constituent of blood[5]. Several factors such as species, breed, sex, age, nutrition, illness, stress, exercise, transportation, and seasonal variations can affect the haematological and biochemical profiles[6]. The present analyses are, thus, conducted to develop a standard reference value of different possible haematobiochemical and cytomorphometrical parameters for both male and female studied species of the nature.

A good number of publications pertaining to the haematology of squirrel is unavailable in record. Therefore, the cytological and biochemical parameters of the circulating fluid like blood of *F. palmarum* are focused in this study. Blood is a connective body fluid that transports necessary substances to the cells of body like nutrients, oxygen, and also excrete waste products. It can act as a pathophysiological indicator of animals' health. Experimental study and observations have proved that these may be used as indicators of diseases or stress in animals. Therefore, it is pertinent to find out the normal ranges of haemocytological and biochemical parameters, since pathophysiological processes influence the metabolism and alter the results in experimental protocols.

Materials and methods

For this study, the squirrels were collected by wooden and wire net trapping from Nilagiri (21.46°N 86.77°E) of district

Balasore, Odisha, India. Adult animals of both the sexes of same weight, same place, and same season were taken for the experiment. One ml of blood from each individual was collected by jugular venepuncture and were stored in vacutainer containing K_3 ethylenediaminetetraacetic acid (EDTA) (Kj EDTA, 2ml 13mmx75mm, Mfg By-HXS Tech Co., Limited. PRC. For- Peerless Biotech Private Limited, Chennai, Tamil Nadu, India). These samples were collected between 7 am to 9 am to reduce the variation in blood constituent caused by the biological rhythm. The vials were placed in self-devised chilled box to avoid denaturation or degradation of proteins. This was followed by the analysis of the parameters. The amount of haemoglobin was measured by Sahli's haemoglobinometer, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated as per the following formulae. i. Mean corpuscular volume is equal to packed cell volume upon Red Blood cell multiplied by 10. ii. Mean corpuscular haemoglobin is equal to haemoglobin upon Red blood cell multiplied by 10. iii. Mean corpuscular haemoglobin concentration is equal to haemoglobin upon packed cell volume multiplied by 100.

At 3, 500 revolutions per minute the microcapillary tube was centrifuged for 20 minutes and the haematocrit values were observed in a microhaematocrit reader. The morphometric calculation of the cellular and nuclear length and breadth of erythrocytes, and leucocytes (monocytes, lymphocytes, neutrophils, eosinophils, and basophils) were undertaken. With the help of 20 mm Dispo Van neddle (Manufactured by Hindustan syringes and Medical devices, Faridabad), the blood was drawn and thin smears were prepared on clean grease free plain glass slides (Riviera T^M Cat. No 72900135 measuring 76.2 mm x25.4mm x1.2mm, West Bengal). For each sample, the smears were prepared to observe the cells of leucocytes and erythrocytes along with the morphometry of those cell. The slides with blood smear were then fixed in absolute methanol (a product of Nice Chemicals Pvt. Ltd, Kerala) for five minutes, followed by staining with Leishman's stain (a product of Nice Chemicals Pvt. Ltd, Kerala). The percentage of eosinophils, neutrophils, lymphocytes, monocytes, and basophils was enumerated under a light microscope (Olympus, CH20i, Olympus Opto Systems India Pvt. Ltd. Noida) as per the standard procedure of some workers[7,8]. Cells were classified and expressed in percentage [9]. One thousand erythrocytes per slide were counted and four slides per squirrels were taken into account for counting. Thirty cells of each cell type for each specimen in each sex were measured with the help of microscope having attachment of both the equipments like camera (CatCam130- 13 Mega Pixel, Code No. CC130, Catalyst Biotech, Maharashtra, India) attached to Hund Wetzlar Microscope (GmbH, Wetzlar Nauborn, Germany) and computer (Compaq CQ3120IX, USA).

For biochemical analyses, the sample was centrifuged at 3,000 revolution per minute for 10 minutes and the serum was placed at -8℃. The biochemical parameters such as glucose, total protein, globulin, albumin, and cholesterol were estimated using commercial kits (Coral Clinical System, US Nagar, Uttarakhand, India).

Statistical analysis: The mean values (M±SE) of the haematological parameters were calculated and the effect of sex on these parameters were also analysed using T- test[10].

Results and discussion

In this investigation, the haematological, biochemical, differential leucocytes count, and morphometric analyses have been taken into account. The results of this finding reveal the blood profile for both the sexes of *F. palmarum*. The entire data of haematology are computed and tabulated (Table-1). The per cent of RBC is found to be 4.27±0.18 in males and 2.2±0.13 in females. Whereas WBC in both the sexes is 41.0 ±0.81 and 21.4±0.88, respectively. The packed cell volume (PCV) content is 21.4±0.88 and 8.33±0.09 for both the sexes of the squirrel. The per cent of haemoglobin for different sexes is 8.33±0.09 and 6.81±0.20, respectively. The value of MCV is recorded to be 19.06±8.34 in males and 32.73±170.51 in females. The value of MCH is 10.08±3.14 and 10.65±0.40 in males and females, respectively. Further, the value of MCHC of males and females is 20.39±0.48 and 32.27±1.38, respectively.

Table-1: Haeamatological parameters of the male and female squirrels (n=05 per sex).

Haematological parameters	Sex of *F. palmarum*	
	Male	Female
RBC (10^6 mm-3)	2.2±0.13***	4.27±0.18***
WBC (10^3 mm-3)	41±0.81***	21.4±0.88***
Hb (g/dl)	8.33±0.09***	6.81±0.20***
PCV (%)	8.33±0.09***	21.4±0.88***
MCV (fl)	19.60±8.34***	32.73±170.51***
MCH (pg)	10.08±98.67^{NS}	10.65±1.61^{NS}
MCHC (%)	20.39±2.35***	32.27±19.2***

The biochemical components such as glucose, cholesterol, albumin, globulin, and total protein are recorded (Table-2). The value of total protein in males and females is 1.74±0.30 and 0.57±0.15, respectively. The percentage of glucose in both the sexes is 58.26±11.65 and 43.70±6.66, respectively. The value of cholesterol in males and females is 24.70±3.83 and 15.48±2.03, respectively. The albumin content has been noted to be 2.40±0.41 in males and 1.21±0.33 in females. The observed value of MCHC is found to be 20.39±2.35 in males and 32.27±19.20 in females.

Table-2: Biochemical parameters of the male and female squirrels (n =05 per sex).

Biochemical parameters	Sex of *F. palmarum*	
	Male	Female
Total protein (g/dl)	1.74±0.30***	0.57±0.15***
Cholesterol (mg/dl)	58.26±11.65**	43.70±6.66**
Glucose (mg/dl	24.70±3.83^{NS}	15.48±2.03^{NS}
Albumin (g/dl)	2.40±0.41**	1.21±0.33**
Globulin (g/dl)	0.86±0.21^{NS}	0.62±0.15^{NS}

The results of differential leucocyte count in percentage are expressed in detail (Table-3). The value of lymphocytes of males and females is 29.7±2.61 and 23.9±1.04, respectively. The value of monocytes is calculated to be 14±0.81and 14.7±1.01 in males and females, respectively. The percentage of neutrophils of males and females is 51.1±1.73 and 52.7±2.19, respectively. The value of eosinophils in males and females is reflected to be 1.4±0.16 and 1.5±0.22, respectively. The figure of basophils of males and females are 0.4±0.16 and 0.5±0.16, respectively.

The findings of morphometric analyses in micron (μ) of *F. palmarum* are noted (Table-4). The cellular length and breadth of erythrocytes of both males and females are 6.33±0.11, 9.81±0.20 and 5.72±0.13 and 9.61±0.20, respectively. The length and breadth of both cells and nuclei of lymphocytes of males and females are 8.61±0.33, 7.86±0.27, 10.18±0.20, 10.02±0.20, and 7.71±0.30, 7.37±0.28, 9.55±0.15, 9.35±0.15, respectively. The monocytes' length and breadth are 8.50±0.46 and 8.68±0.24 between males and females, whereas that of breadth between the sexes is 7.41±0.37 and 9.09±0.22. The cell length and breadth of neutrophils of males and females are 8.87±0.20, 9.09±0.22, and 8.11±0.20, 8.77±0.23, respectively. The cell length and breadth of eosinophils are 7.74±0.31, 7.99±0.26, and 7.26±0.34, 9.64±0.29 between males and females, respectively. Moreover, the length and breadth of basophils in males as well as females are 9.14±0.36, 9.14±2.6, and 8.42±0.38, 9.64±1.73, respectively.

Discussion: In this investigation, the adult male and female squirrels were considered for the study. The findings of haematological, biochemical, differential leucocyte count, and morphometrical analyses of healthy *F. palmarum* can be taken as a reference value for the pathological observation. The RBC is greater in males than females which might be due to the variation in sex which differs significantly by $p<0.001$. RBC is involved in the transportation of O_2 and CO_2 in the body[2]. Reduction of red blood cell indicates less amount of oxygen in body[11,12]. The key role of the white blood cells is to eliminate foreign particles by the process of phagocytosis and the production, transportation, and distribution of antibodies. The value of white blood cell is higher in males than females, with a significant difference by p less than 0.001. The higher value of WBC in males may be due to the infection caused due to pathogens like bacteria, fungi, and parasites.

Table-3: Cytomorphometrical analyses of male and female squirrels (n=05 per sex).

Types of Cells	Cell/Nucleus	Prameters	Sex of *F. palmarum*	
			Male	Female
Erythrocyte	Cell	Length Breadth	6.33±0.11*** 5.72±0.13***	9.81±0.20*** 9.61±0.20***
Lymphocyte	Cell Nucleus	Length Breadth Length Breadth	8.61±0.33*** 7.86±0.27*** 7.71±0.30*** 7.37±0.28***	10.18±0.20*** 10.02±0.20*** 9.55±0.15*** 9.35±0.15***
Monocyte	Cell	Length Breadth	8.50±0.46^{NS} 7.41±0.37*	8.68±0.24^{NS} 9.09±0.22*
Neutrophil	Cell	Length Breadth	8.87±0.20** 8.11±0.20**	9.09±0.22** 8.77±0.23**
Eosinophil	Cell	Length Breadth	7.74±0.31^{NS} 7.26±0.34^{NS}	7.99±0.26^{NS} 9.64±0.29^{NS}
Basophil	Cell	Length Breadth	9.14±0.36** 8.42±0.38**	8.42±2.93^{NS} 9.41±2.07^{NS}

Table-4: Mean ±SE Differential leucocyte count of male and female squirrels (n=05 per sex).

Leucocytes	Sex of *F. palmarum*	
	Male	Female
Lymphocyte	29.7 ± 2.61^{NS}	23.9 ± 1.04^{NS}
Monocyte	14 ± 0.81^{NS}	14.7 ± 1.01^{NS}
Neutrophil	51.1 ± 1.73^{NS}	52.7 ± 2.19^{NS}
Eosinophil	1.4 ± 0.16^{NS}	1.5 ± 0.22^{NS}
Basophil	0.4 ± 0.16^{NS}	0.5 ± 0.16^{NS}

Haemoglobin plays a major role to transfer oxygen for the oxidation of ingested food. For other functions of the body as well as transport of carbon dioxide from the body, haemoglobin helps in delivering energy[13,14]. In this investigation, the per cent of haemoglobin in males and females is 8.33±0.09 and 6.81±0.14, respectively. The haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) or packed cell volume (PCV) is the percentage (%) of red blood cells in blood[15]. The value of PCV is higher in males in comparison to the females. This is probably owing to the greater number of RBC in males which has been observed to be significantly different at $p<0.001$. The value of MCV is higher in females in comparison to males, which is calculated to be insignificant. The value of MCH and MCHC is higher in males than females, which may be possible for the difference in sex or greater value of RBC in males. The value of MCHC differs significantly by $p<0.001$ and MCH does not show any significant difference. The principal factors to evaluate circulatory erythrocytes are packed cell volume, haemoglobin, and mean corpuscular haemoglobin which are significant to diagnose the anaemic nature of the animals. These parameters help as effective indicators of the bone marrow to make red blood cells as in mammals[16,17].

In biochemical analyses, the percentage of protein is higher in males than females which is significantly different by p less than 0.001. The value of protein is higher in males in comparison to females which is expected to be due to the variation in sex or activities of the males. Males show higher concentration of cholesterol than females is significantly different at $p<0.05$. The value of cholesterol is higher in males than females is apparently owing to the difference in sex. The content of albumin is greater in males than females and it differs by $p<0.01$. This may be due to the rich content of protein in males than females. The percentage of globulin is greater in males in comparison to females, which also exhibits significant difference at p less than 0.05. The greater value of globulin in males than females may be due to the variation in sex or higher value of protein of male sex.

Differential leucocyte count (DLC) can be used as a noteworthy component to ascertain the health, resistance against genetic disease, stresses due to environmental, nutritional, and pathological factors. In this study, the number of lymphocytes shows higher in males than females, which is believed to be due to the variation in sex. The per cent of monocytes, neutrophils, eosinophils, and basophils is higher in females than males, which is because of variation in sex or some infections. The lymphocytes, monocytes, neutrophils, eosinophils, and basophils do not show any significant difference in both the sexes.

Blood cells morphometry, a salient feature of haematology, can regulate the bodily functions of the organisms. In this investigation, the length and breadth of erythrocytes, monocytes, eosinophils, and basophils show higher values in females than males, which differ significantly at $p<0.001$. The length and breadth of erythrocytes differ significantly at $p<0.001$, showing higher values in females than males. The erythrocytic length and breadth differ significantly at $p<0.001$. The length and breadth of both cells and nuclei of lymphocytes are higher in females in comparison to males, which again differ at $p<0.001$. The cellular length and breadth of monocytes are more in females than males, which differ at $p<0.05$. This significant difference is due to the variation in sex. The cell length and breadth of neutrophils is observed to be higher in female individuals than that of males, which differ significantly by $p<0.05$. The cell length and breadth of eosinophils are higher both in females and males and that of basophils are also higher in both the individuals. Interestingly, the eosinophils as well as basophils do not show any significant difference. The results of this investigation corroborate with the findings of other researchers[18,19].

Figure-1: *F. palmarum* in natural habitat.

Figure-2: Trapped *F. palmarum* in a wire net cage.

Figure-3: Anesthetized *F. palmarum* on a tray.

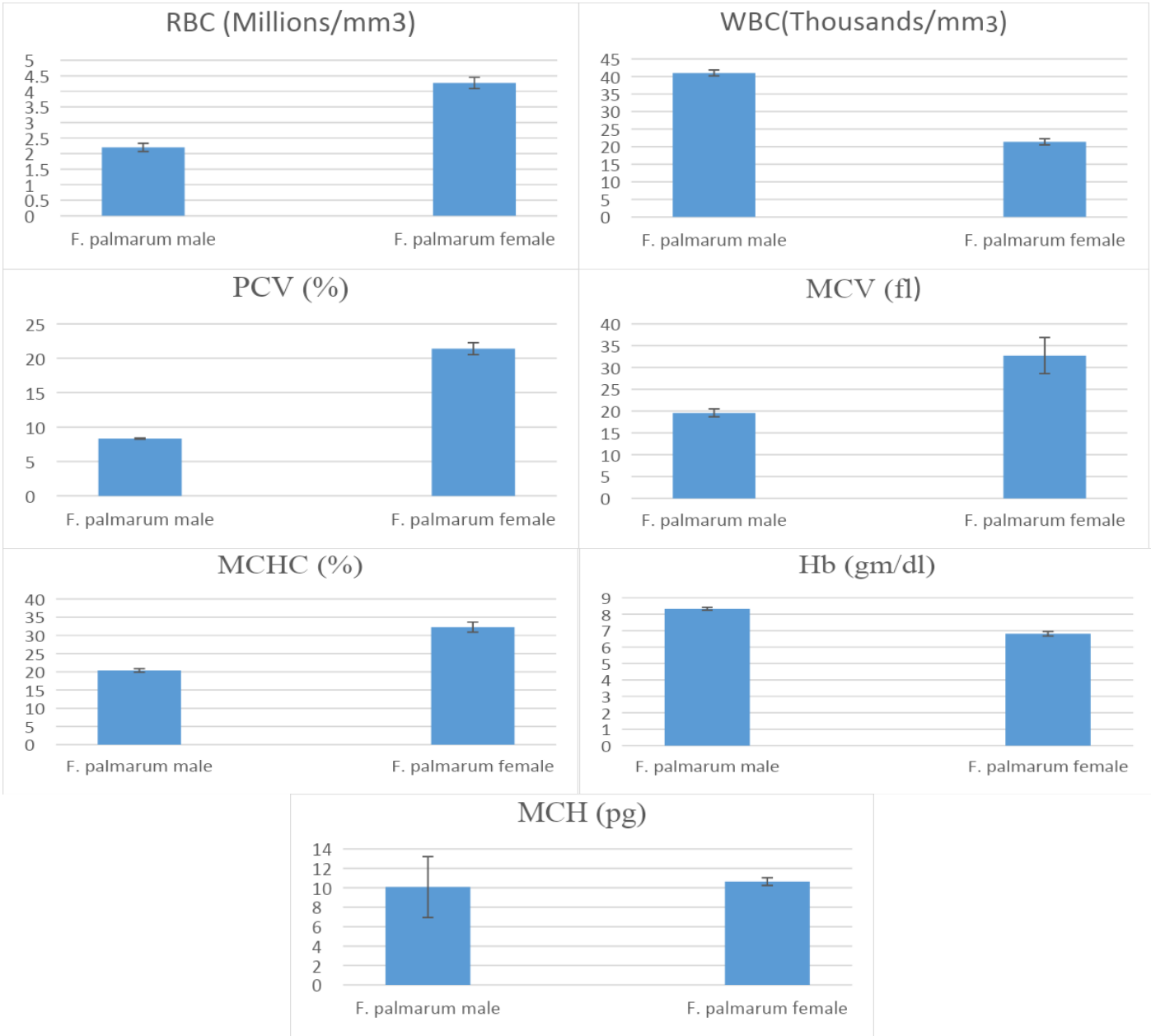

Figure-4: Haematological parameters of *Funambulus palmarum*.

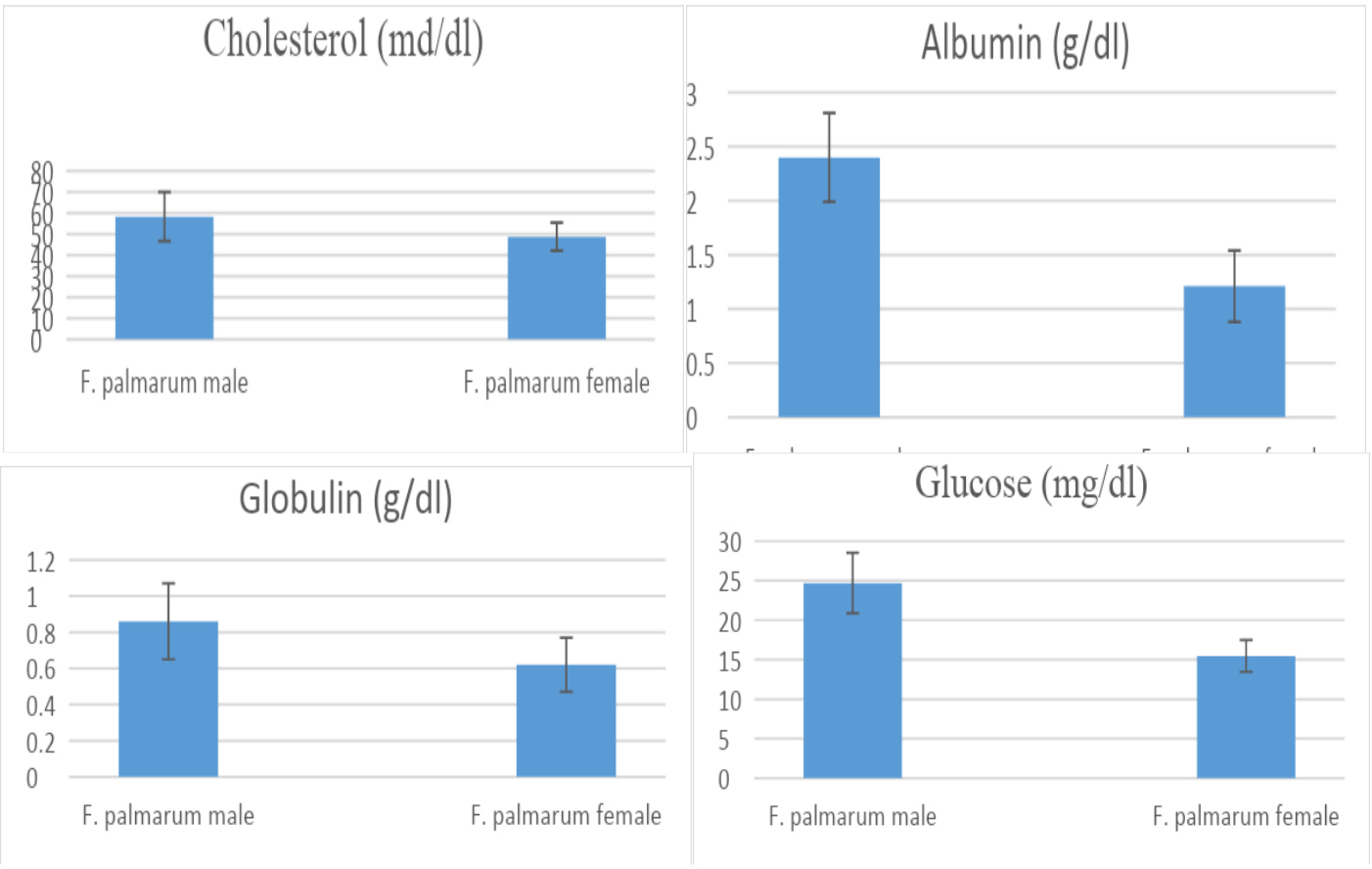

Figure-5: Biochemical parameters of *Funambulus palmarum*.

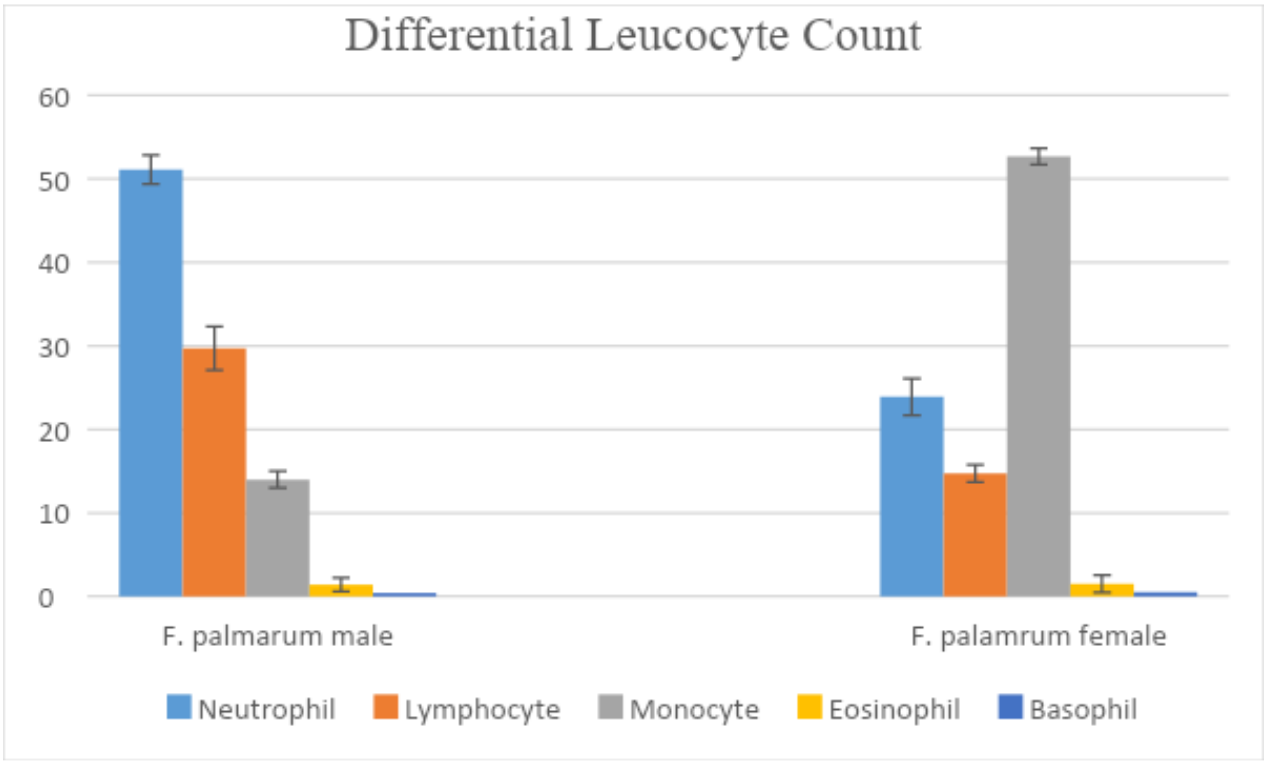

Figure-6: Differential Leucocyte count of *Funambulus palmarum.*

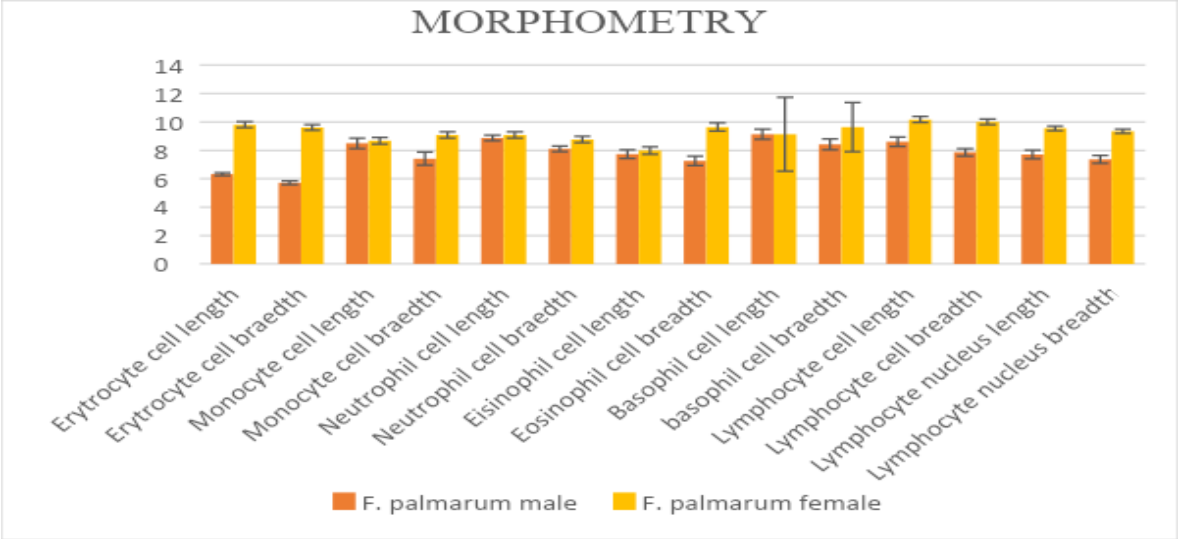

Figure-7: Morphometrical analyses of *Funambulu palmarum.*

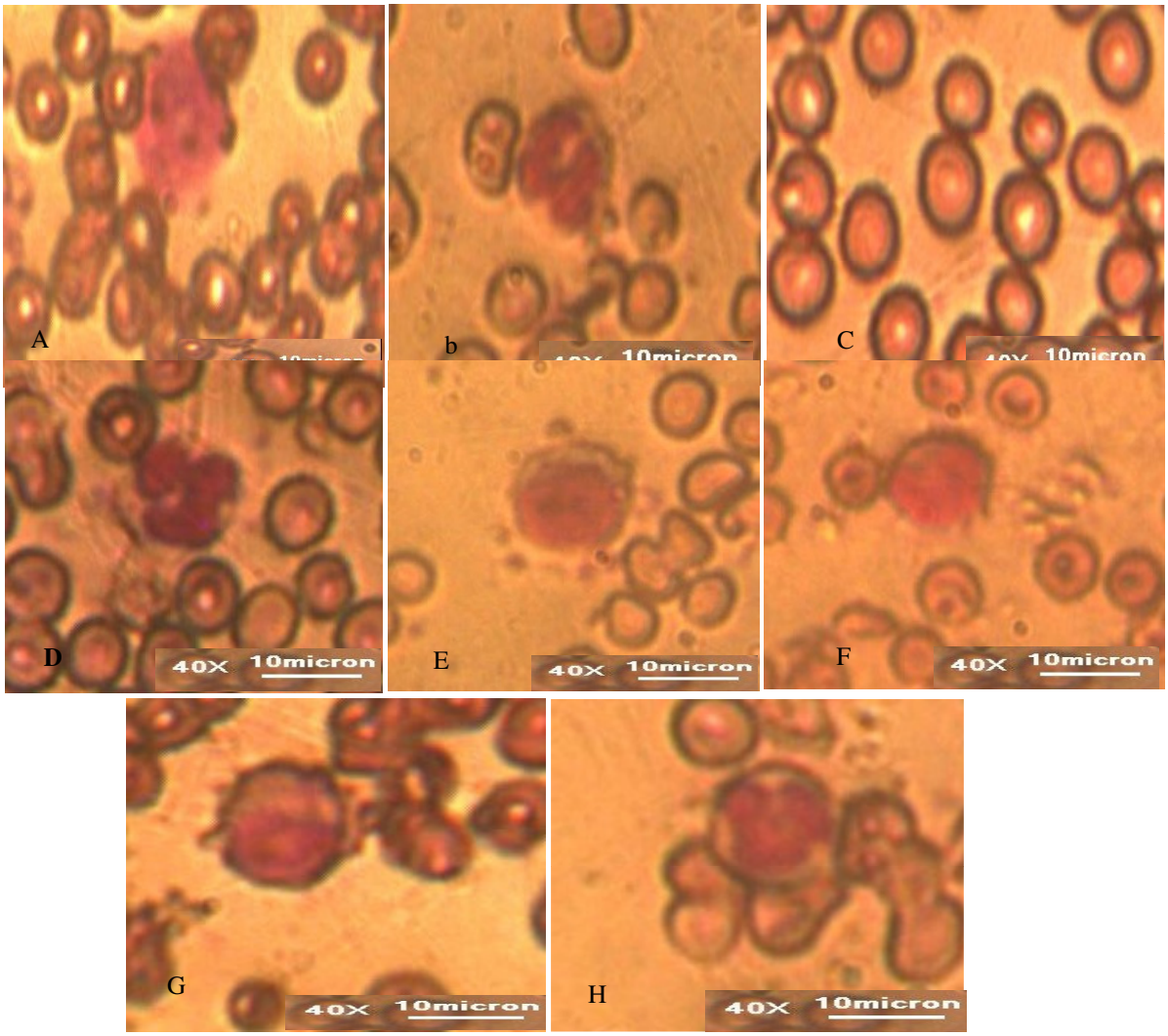

Figure-8: Different Blood Cells of *F. palmarum* : A. Basophil, B. Eosinophil, C. Erythrocyte, D. Flower shaped Neutrophil, E. Large Lymphocyte, F. Lymphocyte, G. Monocyte, H. Neutrophil.

Conclusion

The investigation reveals the haemocytobiochemical and cytomorphometrical condition of Indian palm squirrel, which are different due to the effect of the sex. The results of the present haematological and biochemical study would help in pathological diagnosis, treatment, protection, and developing healthy generation in future.

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