

SEX-SPECIFIC VARIATION IN COMPLETE BLOOD COUNT
PARAMETERS AND BIOMETRIC TRAITS IN WILD RODENTIA:
IMPLICATIONS FOR HORMONAL REGULATION OF
ERYTHROPOIESIS AND SOMATIC GROWTH

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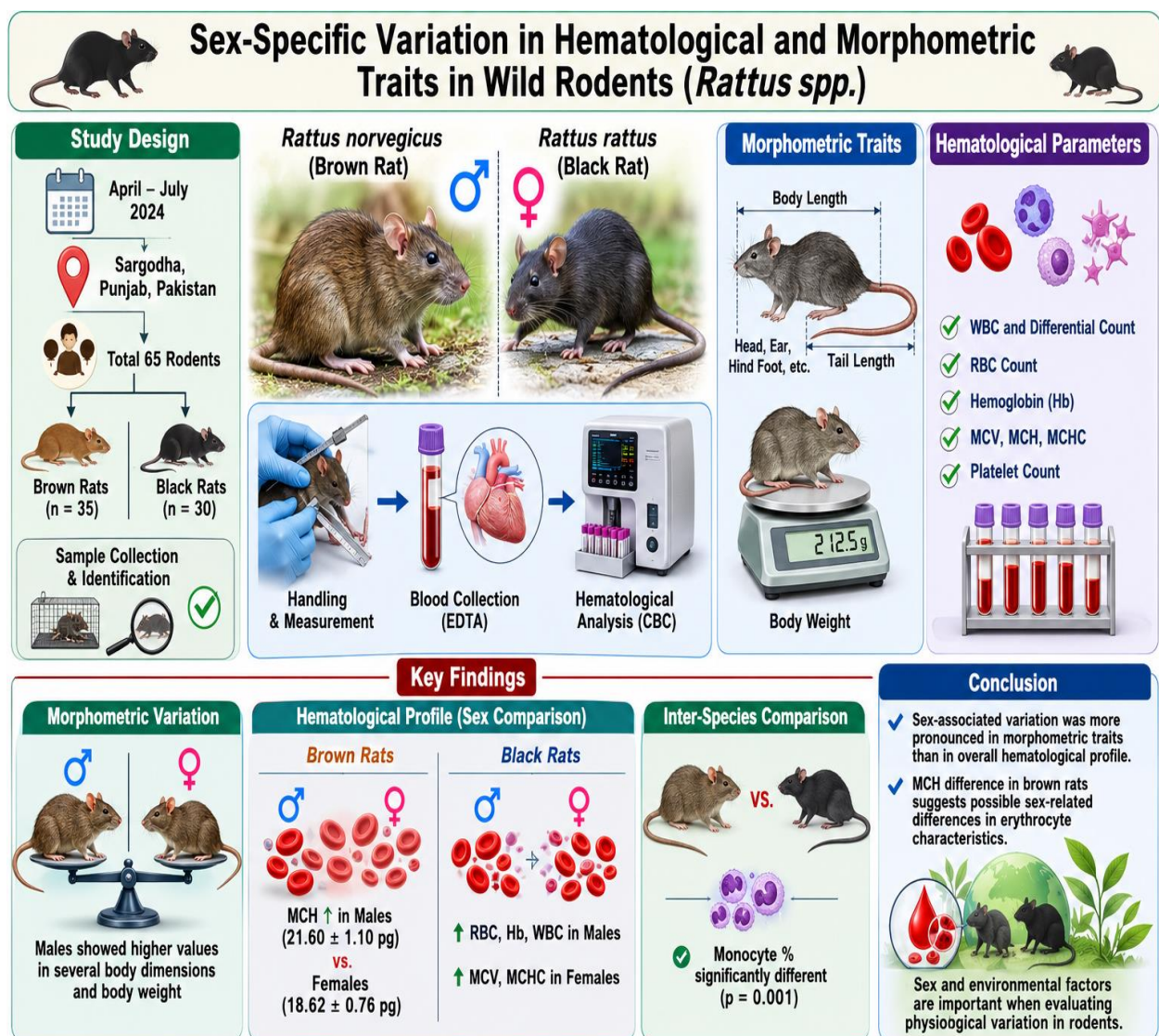
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Abstract

Haematological and morphometric parameters may provide crucial information on the physiological state, immunological status and somatic diversity among wild rodents of different sexes. However, these features may also be affected by the environment and biology. The present study compared the complete blood count (CBC) parameters and morphometric characteristics of wild brown (*Rattus norvegicus*) and black (*Rattus rattus*) rats from Sargodha, Punjab, Pakistan with respect to sex. During April-July 2024, a total of 65 rodents were collected from different localities in Sargodha, of which 35 were brown rats and 30 were black rats. A dichotomous key was used for identification of the specimens, and morphometric characters were measured with a Vernier calliper and digital balance. Cardiac puncture samples were taken in EDTA tubes, and major hematological parameters were measured. A two-sample t test was used to make statistical comparisons in Minitab. The morphometric analysis revealed a significant sexual difference in body weight and various body dimensions, with higher values for some of the body dimensions in males than in females. A comparison of the hematological profile showed few differences between the sexes. There were no significant sex differences in most leukocytic and erythrocytic parameters in brown rats, except for mean corpuscular hemoglobin (MCH), which was significantly higher in males (21.60 ± 1.10 pg) than in females (18.62 ± 0.76 pg; $p = 0.02$). The mean

RBC, hemoglobin and WBC values were higher in the male black rats, while the MCV and MCHC values were higher in the female black rats. The monocyte percentage was significantly different between brown and black rats ($p = 0.001$), and most of the other CBC parameters were comparable. The results suggest that the sex-associated variation was more pronounced in selected morphometric traits than in the hematological profile as a whole. However, the marked variation in MCH difference between brown rats indicates a possibility for sex-related differences in erythrocyte characteristics, but no hormonal, age, reproductive or nutritional factors were directly tested. The results provide background data regarding hematological and biometric data of wild *Rattus* populations in the study area and emphasize the need to account for sex and environmental factors when evaluating physiological variation in rodents.



Graphical abstract for Sex-Specific Variation in Complete Blood Count Parameters and Biometric Traits in Wild Rodentia

Introduction

Rodents are a large and varied group of mammals and are one of the largest orders, representing approximately 40% of all mammalian species (Harvey et al., 2012; Meerburg et al., 2009). Rodents are also an important group for their ecological functions as herbivores, seed dispersers, prey and ecosystem engineers. Squirrels, voles, and other rodents aid in seed dispersal by collecting and caching seeds, some of which will germinate from the ground after the seed has been stored, which thus increases vegetation cover and regeneration (Garrett & Franklin, 1988). The alteration of soil structure caused by burrowing also affects the soil's water holding capacity, nutrient availability, aeration, and water infiltration capacity. As food for carnivorous mammals, birds and reptiles, rodents also have an important role in food chains and food webs. Their ecological versatility is closely related to their feeding ecology (from herbivores to omnivores) and physiological adaptations (to live in different environments) (El-Safi & Peters, 1991).

Another characteristic of rodents is their high reproductive ability. They have short gestation periods, early sexual maturity and multiple litters that can cause their populations to grow exponentially when environmental conditions are favourable (Beyer & Haller, 1993; Gadberry, 2019). Some species are difficult to manage as agricultural and urban pests, and because of these characteristics, they are widespread. Both brown rats (*Rattus norvegicus*) and house mice (*Mus musculus*) are highly synanthropic and are able to take advantage of human food, shelter

and waste resources (Meerburg et al., 2009). Rodents can inflict significant economic losses on crops and agricultural production by destroying and damaging stored foods, damaging agricultural infrastructure, and damaging crops. They can also serve as reservoirs or vectors for zoonotic pathogens, such as leptospirosis, plague, and hantavirus, posing further public health and economic risks (Beyer & Haller, 1993). There is a high degree of inter- and intraspecific variability among species of rodents in morphology, physiology, behavior and ecological adaptation traits.

The brown rat is, in general, larger and stronger, with a shorter tail than the head and body, while the black rat (*Rattus rattus*) is slimmer, has a longer tail and larger ears and is adapted to climbing and living in trees. The differences are significant for proper identification of the species and proper population management. There are also significant differences in social behavior among species of rodents, some relatively solitary, others, such as prairie voles and prairie dogs, which are very social (Hoffmann & Zeller, 2005). Another noticeable difference is the way that some species adapt to different environments; kangaroo rats (*Dipodomys* spp.) in the desert have very efficient water conserving systems, while other species are adapted to cooler or wetter environments.

The physiological condition of rodents and their health status can be determined by hematological parameters. Species, age, sex, nutrition, environment, disease and stress can affect parameters such as red blood cell count,

white blood cell count, hemoglobin, hematocrit and platelets (Clegg et al., 2006; Islam et al., 2021). Thus, haematologic evaluation is broadly applied to assess general rodent health status and to assess responses to environmental contaminants, nutritional changes, and experimental treatments (Shreadah et al., 2017). *Rattus norvegicus* and *Rattus* are two of the most significant rodents due to their close association with people, abundance, high fecundity, and economic and public health importance. Brown rats are often found in sewers, basements, agricultural fields and other low-lying environments, while black rats are often found in high structures and roofs, trees and attics. Both species are sexually dimorphic, with male and female specimens being typically larger and more muscular and females investing more in gestation and lactation, having specialized reproductive structures (Greaves & Kumar, 1998). To accurately assess their ecology, to monitor populations and to manage them successfully, it is important to recognize these differences in terms of morphology, physiology, behaviour and sex. The biology, distribution, hematology and inter- and intraspecific variation of rodents, therefore, provides vital insights into their roles in agriculture, economics and public health as well as their ecological roles.

The primary aim of this study was to investigate sex-associated variation in the morphometric and hematological characteristics of wild brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) inhabiting Sargodha, Punjab, Pakistan. Specifically, the study aimed to (i) characterize and compare major biometric traits between

male and female rats, (ii) evaluate sex-related differences in complete blood count parameters, including leukocytic and erythrocytic indices and platelet counts, (iii) compare hematological profiles between *R. norvegicus* and *R. rattus*, and (iv) determine whether observed differences provide evidence of sex-associated physiological and morphological variation in these wild rodent populations. The study design and measurements are described in the manuscript.

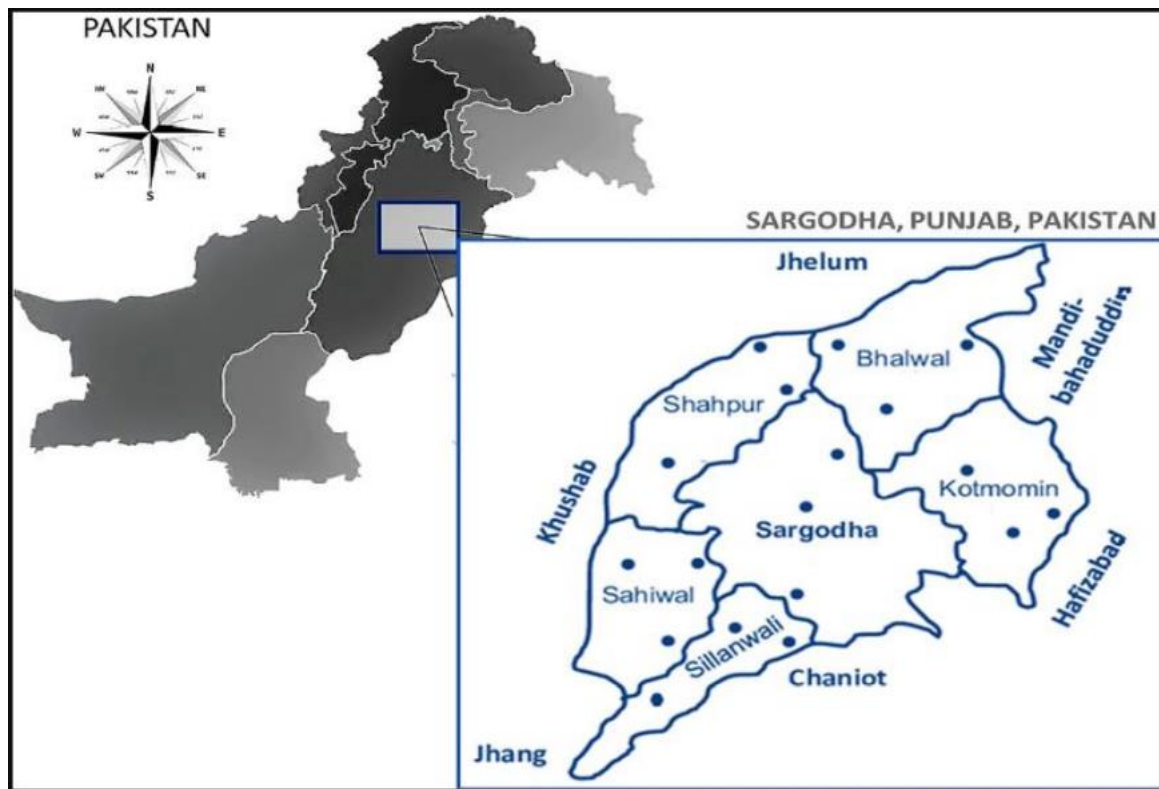
Materials and Methods

Material

Wooden boxes, surgical gloves, EDTA tubes, dissection boxes, Vernier callipers, digital weight balances, insulin syringes, ethanol, droppers, beakers, cotton, and tap water were used at different steps in this study.

Sample collection

Sixty-five samples of rodents were collected from April to July 2024. Among them, 35 were brown rats and 30 were black rats. These rats were collected from various regions of Sargodha, Punjab, Pakistan. A small amount of bait, such as chunky peanut butter or mutton fat, was placed on the snap trap in areas where mice and rats had been observed. In basements, outbuildings and similar places where there had been no regular activity by humans, their nesting materials, urine and feces, food eaten or gnaw marks were noted. After capturing, they were transported to the Zoology Laboratory, The University of Lahore (Sargodha Campus) and kept alive by providing sufficient food and drinking water.



Study area map

Species Identification

Collected samples were identified using an available key (dichotomous key). Two species of rodents, black rats (*Rattus rattus*) and brown rats (*Rattus norvegicus*), were identified.

Identification key for brown rat (*Rattus norvegicus*)

One of the most widely distributed species of rat is the brown rat (*Rattus norvegicus*). They weighed between 140 and 500 g (5 and 17+3.4 oz.). It is one of the largest muroids and is characterized by its brown to brownish-grey colour. The brown rat has a pointed nose, large bare ears, and a long scaly tail. Compared to male rats, female brown rats are slightly smaller and less in weight (Figure 1a & 1b)

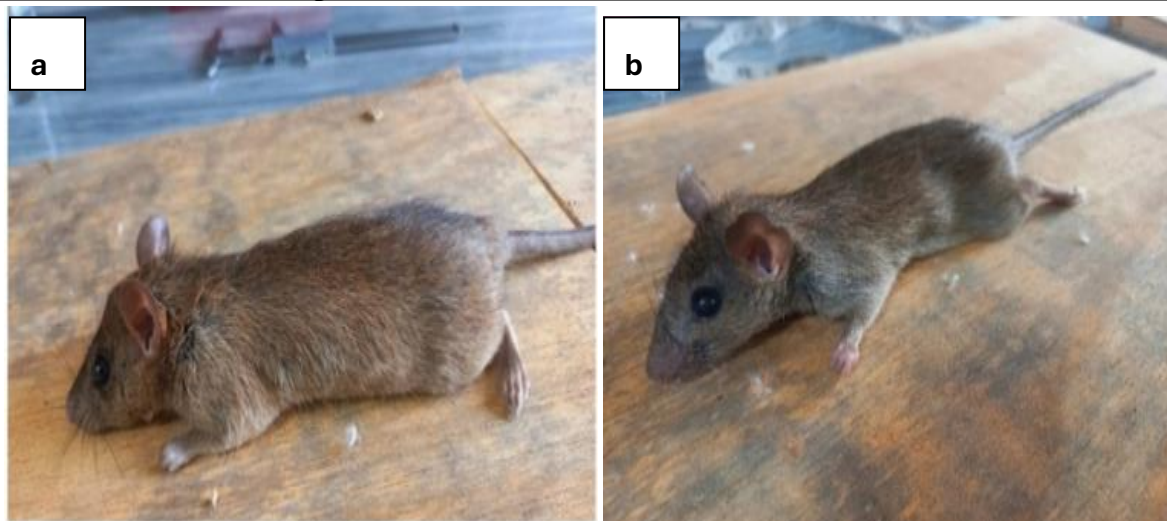


Figure 1: Male brown rat (a) and female brown rat (b)

Identification key for black rat (*Rattus rattus*)

The tail of a *Rattus rattus* is significantly longer than its head and body. The underbelly of black rats is typically lighter in color, ranging from black to light brown. Additionally, they are slightly smaller than Brown rats and have a

scraggly black fur coat. Additionally, they can be found in agricultural settings such as barns and crop fields. Because they preferred to live in dry upper levels of buildings in urban areas, they are frequently discovered in false ceilings and wall cavities (Figure 2a & 2b).

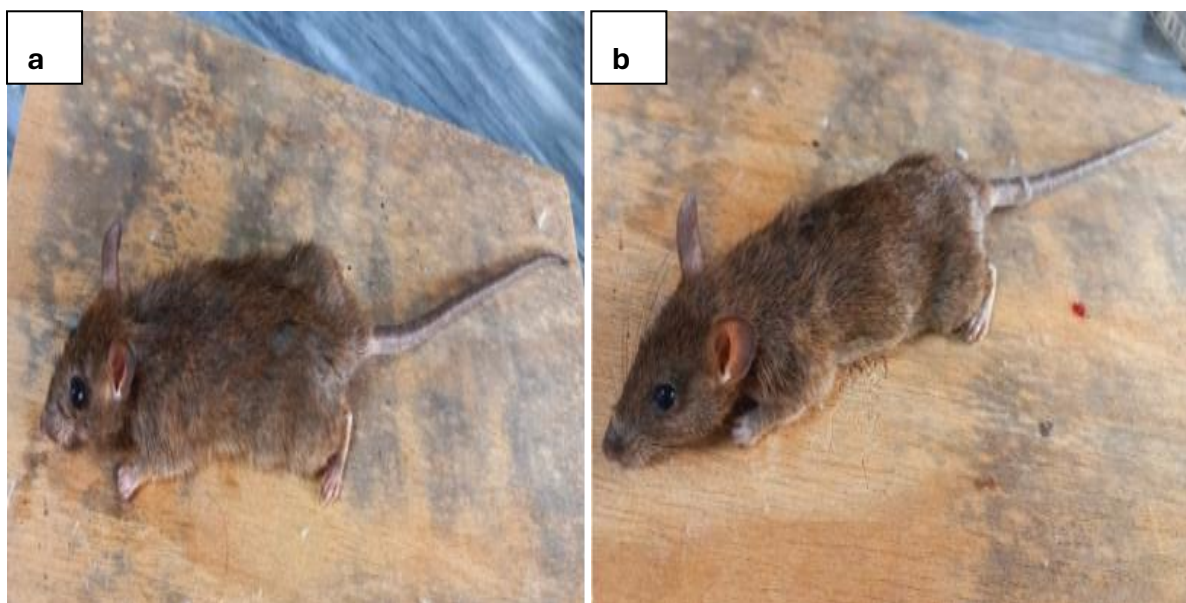


Figure 2 (2a-2b): Male black rat (a), female black rat (b)

Morphometry of samples

First, morphometry of all the samples was performed with the help of a Vernier calliper. Morphometry included head and body length, head length, tail length, snout length, eye length, head width, hind foot length, ear length, body width, body girth, and front foot length.

Blood collection

Blood was collected from the heart puncture with the help of an insulin syringe. Blood was transferred into screw-capped tubes containing 0.5 ethylene diamine tetra acetic acid (EDTA) solution as an anticoagulant for complete blood count CBC analysis (Figure 3).



Figure 3: Blood collected through insulin syringe

CBC Blood Test

A complete blood count, or CBC, is a blood test that includes red platelets, white platelets, platelets, hemoglobin, and mean corpuscular volume (MCV). In gender-specific differences,

males have higher red blood cell counts and hemoglobin concentrations, while females typically exhibit elevated white blood cell counts, reflecting stronger immune responses. These differences can affect disease

susceptibility, including infections, anemia, and hematological cancers, and underscore the importance of considering sex in studies of rodent health.

Morphological analysis

In this study, gender-specific differences in morphometric parameters of wild rats were assessed by measuring body weight, body length, and organ size. Male and female rats were captured from the habitat, and their physical characteristics were recorded using standardized callipers and a digital scale. The data were analysed to compare the size dimorphism between sexes and to focus on variations in overall body size and organ.

Statistical Analysis

All morphometric and hematological data were statistically analysed using Minitab. Differences between groups were evaluated using a two-sample independent t test. The results are expressed as the mean $\pm$ standard error (SEM) where applicable, and statistical significance was considered at $p < 0.05$. For hematological comparisons between brown and black rats, individual parameters, including WBC, differential leukocyte counts, RBC, hemoglobin, MCV, MCH, MCHC, and platelet counts, were subjected to the two-sample t test. The manuscript reports that most parameters

did not differ significantly between species, whereas monocyte percentage showed a significant difference ($p = 0.001$).

Results

Morphometry of Brown Rat

Tables (1) and (2) show the maximum and minimum values of different parameters measured, such as head body length (HBL), head length (HL), tail length, body weight (BW), hind foot length, ear length, head width, snout length, body width, body girth, eye diameter, front foot, total length and the average of these parameters for female and male brown rats separately.

In Table (1), the maximum length of the female brown rat was 41.7 cm, and the minimum was 28.2 cm, whereas the head body length and tail length range of the female brown rat were 21.3 cm and 13.9 cm and 20.6 cm and 14.4 cm, respectively. The maximum and minimum weights of female brown rats are 169.5 g and 39.9 g, respectively.

In table (2), the maximum length of male brown rats was 45.1 cm, and the minimum was 26.2 cm. Similarly, the body width and body girth range of male brown rats are 5.3 cm and 2.2 cm and 15 cm and 11.3 cm, respectively. The maximum and minimum weights of male brown rats are 233.1 g and 88 g, respectively.

Table 1: Morphometric parameters of Female Brown Rat (*Rattus norvegicus*)

Morphometry	HB L	H L	TL	B W	HF L	EL	H W	S L	B W	BG	E D	FF	TL	Species
Maximum	21	5	20.6	170	3.4	2.2	2.4	0.9	5.1	14.7	0.7	1.7	41.7	Brown rat (female)

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Minimum	14	3.8	14.4	39.9	2.9	2.1	1.8	1.1	2.5	8.7	0.6	1.2	28.2	Brown rat (female)
Average	18	4.5	16.6	10.6	3.01	2.31	2.1	0.9	3.35	11.9	0.6	1.54	35	

Table 2: Morphometric parameters of male brown rats (*Rattus norvegicus*)

Morphometry	HB L	H L	TL	B W	HF L	E L	H W	SL	B W	BG	E D	FF	TL	Species
Maximum	23	5.3	22.3	233	3.1	2.5	2.7	0.6	5.3	15	0.5	1.7	45.1	Brown rat (male)
Minimum	14	4.4	12.6	88	3	2.4	1.8	1.2	2.2	11.3	0.5	2.1	26.2	Brown rat (male)
Average	18	4.3	17.2	126	3.06	2.3	2.2	0.9	3.65	12.2	0.6	1.5	35.6	

Intraspecies comparison of Brown rats (*Rattus norvegicus*)

Male and female *Rattus norvegicus* (brown rats) have significant physical characteristics, as shown in Tables (1) and (2) above. Males often have larger bodies, weighing between 200 and 500 grams, in contrast to females, who typically weigh between 250 and 350 grams. In addition, the tail length of males is typically longer than that of females, ranging from 20 to 45 cm.

Morphometry of Black Rat (*Rattus rattus*)

Tables (3) and (4) show the maximum and minimum values for various parameters, such as head body length (HBL), head length (HL), tail length, body weight (BW), hind foot length,

ear length, head width, snout length, body width, body girth, eye diameter, front foot, and total length. We also calculated the average of these parameters separately for female and male black rats.

In table (3), the maximum and minimum lengths of female black rats are 39.2 and 25.5 cm, respectively. The female black rat's head body length and tail length range are 19.1 cm and 11.4 cm and 20.1 cm and 14 cm, respectively. The female black rat has a maximum weight of 101.2 g and a minimum weight of 27.5 g. In Table (4), the maximum and minimum lengths of male black rats are 39.2 and 25.5 cm, respectively. The male black rat body weight and body width range are 102 g and 85 cm and 4.1 cm and 3.1 cm, respectively.

Table 3: Morphometric parameters of the female black rat (*Rattus rattus*)

Morphometry	HB L	HL	TL	B W	HF L	E L	H W	S L	B W	BG	E D	FF	TL	Species
Maximum	19	4.1	20.1	101	3.4	1.8	2.2	1.2	3.9	13.1	0.8	1.1	39.2	Black rat (female)

Minimum	11	3.7	14	27.5	2.8	2	1.7	0.7	1.9	6.2	0.6	1.1	25.5	Black rat (female)
Average	15	3.91	16	75.3	2.9	2.1	1.9	0.9	3.1	10.8	0.6	1.19	31.8	

Table 4: Morphometric parameters of Male Black Rat (*Rattus rattus*)

Morphometry	HB L	H L	TL	B W	HF L	EL	H W	S L	B W	BG	E D	F F	TL	Species
Maximum	17	4.2	18.5	102	3.1	2.9	1.9	0.8	4.1	8.9	0.5	1.5	35.8	Black rat (male)
Minimum	12	3.5	14	85	2.9	2.2	2.5	0.7	3.1	10.5	0.7	1.2	26.1	Black rat (male)
Average	15	3.6	16.5	97	2.65	2.09	2.1	0.6	2.9	10.5	0.6	1.3	31.2	

Intraspecies comparison of black rats

As previously mentioned in tables (3) and (4), there are significant physical differences between males and females, particularly in body size and weight. Depending on the environment and diet, males typically weigh between 100 and 200 grams and have an average length of 31.2 cm. However, the average female weight was between 70 and 150 grams, and the average length was 31.8 cm.

Interspecies comparison of brown rats and black rats

By using morphometric parameters, we identified significant differences between brown and black rats. The black rat typically has a shorter tail and a smaller body. It has a pointed nose, slim build, and ears that are almost hairless and long. The brown rat is stronger because it has a thicker body, smaller ears that are covered in fine hair, and a blunt snout. The black rat's fur is typically smooth and ranges from black to dark brown, whereas

the brown rat's fur is coarser and typically brown or gray, as mentioned in the tables.

Complete Blood Count

Here, we compared the complete blood count (CBC) of brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*). Several key hematological parameters can be examined to highlight species-specific differences in different regions of Sargodha, Punjab, Pakistan. These parameters typically include white blood cell count (WBC), red blood cell count (RBC), neutrophil, basophil, eosinophil, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, platelet count, etc.

Comparison of CBC between brown rats and black rats

A higher concentration of WBCs (8.60 ± 1.2) was observed in brown rats than in brown rats (6.77 ± 0.64), as shown in Table 5.

Table 5: Comparison of studied complete blood count parameters between brown rats and black rats from Sargodha, Punjab, Pakistan. Data are expressed as the mean $\pm$ standard error of the mean. The P value indicates the result of the 2-sample t test calculated for each studied parameter.

Parameters	Brown rat	Black rat	P value
White blood cells ($\times 10^3 \mu\text{L}^{-1}$)	8.60 $\pm$ 1.2	6.77 $\pm$ 0.64	0.1
Neutrophil %	46.8 $\pm$ 3.0	30 $\pm$ 3.4	0.9
Lymphocytes %	54.4 $\pm$ 3.4	61.0 $\pm$ 3.4	0.1
Monocyte %	34.5 $\pm$ 5.3	12.9 $\pm$ 2.7	0.001***
Eosinophil %	0.52 $\pm$ 0.14	0.49 $\pm$ 0.15	0.8
Basophil %	0.93 $\pm$ 0.16	0.98 $\pm$ 0.14	0.3
Red Blood Cells ($\times 10^6 \mu\text{L}^{-1}$)	5.88 $\pm$ 0.26	5.91 $\pm$ 0.19	0.9
Hemoglobin $\times$ g/dL	10.87 $\pm$ 0.42	10.99 $\pm$ 0.35	0.9
Mean corpuscular volume (μm^3)	53.5 $\pm$ 1.8	54.75 $\pm$ 1.6	0.6
Mean corpuscular hemoglobin (pg)	19.92 $\pm$ 0.67	20.69 $\pm$ 0.81	0.4
Mean corpuscular hemoglobin concentration (gdl $^{-1}$)	33.32 $\pm$ 0.96	32.99 $\pm$ 0.80	0.7
Platelets ($\times 10^3 \mu\text{L}^{-1}$)	455 $\pm$ 37	420 $\pm$ 27	0.4

The P value shows a nonsignificant difference, whereas the concentration of monocytes in black rats becomes higher (12.9 $\pm$ 2.7) than that in brown rats (34.5 $\pm$ 5.3). The P value becomes highly significant.

White Blood Cell and Differential Leukocyte Parameters in Brown Rats

The total white blood cells (WBCs) and percentages of the differential leukocytes were evaluated in brown rats and were generally found to be similar between sexes, with lymphocytes and neutrophils being the most important leukocytes. Total white blood cell (WBC) counts were nearly the same, averaging approximately 8.6 for females and 8.7 for males. Lymphocytes made up the highest percentage of leukocytes for both groups of females (55.2%) and males (53.5%), with neutrophils next at 45.4% (showing significant sample variation) and 48.7%, respectively. A moderate difference in percentages between males and females was found for monocytes at 37.5% and 32.3%, respectively. In minor leukocytes, eosinophils were consistently low (0.5% in both sexes) and basophils (1.2% females and 1.1% males) (Figure 7a).

Red blood cell parameters in black rats

The present study showed that there were subtle sex-specific differences in some of the key parameters in studying the erythrocytic indices of black rats. Male black rats had slightly higher red blood cell (RBC) counts (6.1) and hemoglobin concentrations (11.3 g/dL) than females (5.7 and 10.8 g/dL, respectively). However, females had higher mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) mean values of 57.6 fL and 21.9 pg, respectively, than males, who exhibited values of 50.9 fL and 19.1 pg, respectively. The mean corpuscular hemoglobin concentration (MCHC) was 33.1% among females and 32.7% among males, which is comparable to each other (Figure 7b).

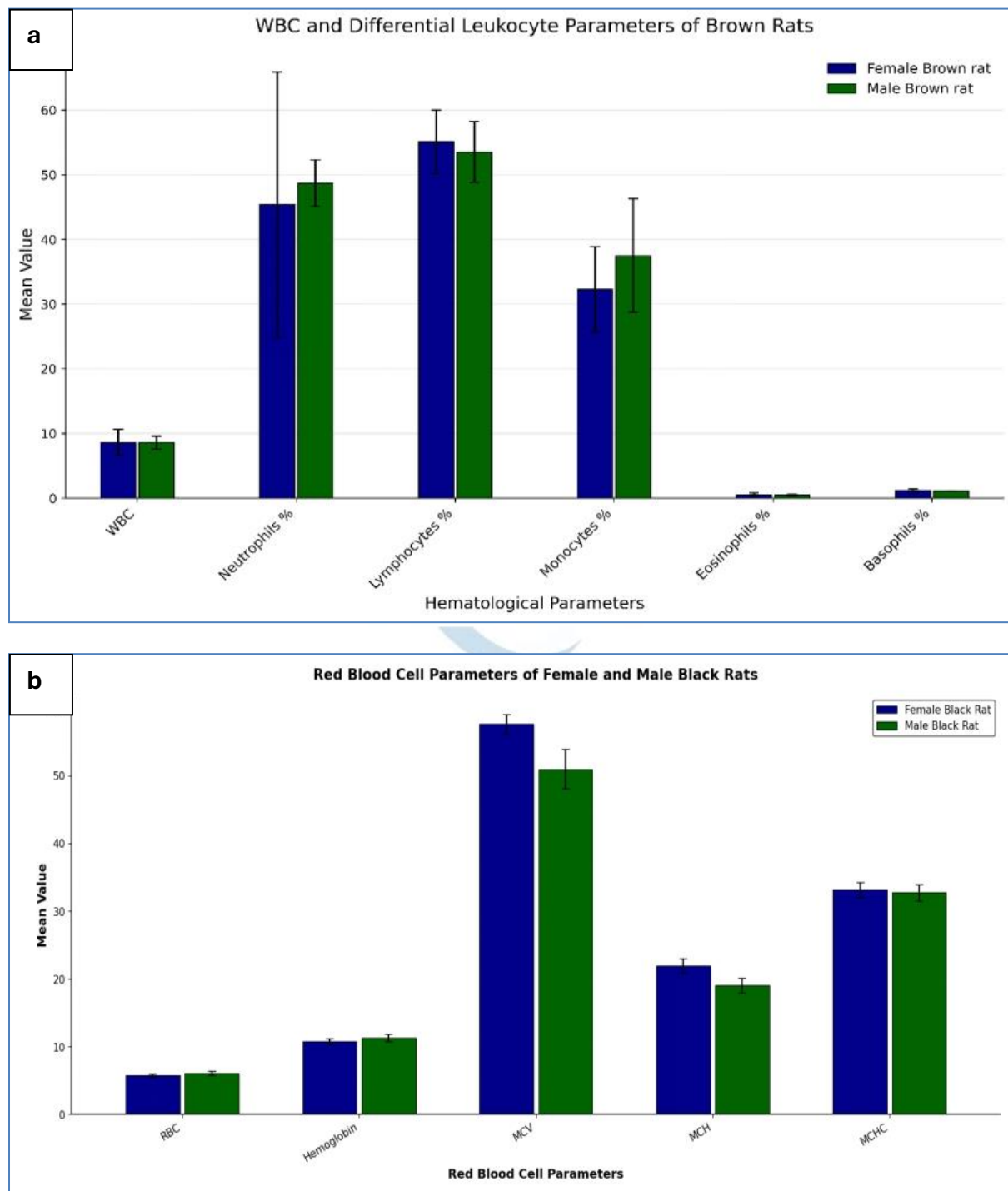


Figure (7a-7b) a) White blood cell (WBC) count and differential leukocyte percentages (mean $\pm$ SD) in female and male brown rats b) Red blood cell (RBC) parameters (mean $\pm$ SD) in female and male black rats.

Red blood cell parameters of black rats

The erythrocytic indices were analysed in black rats, and each parameter exhibited a different profile between sexes along with their cumulative profile. The mean RBC count (6.2

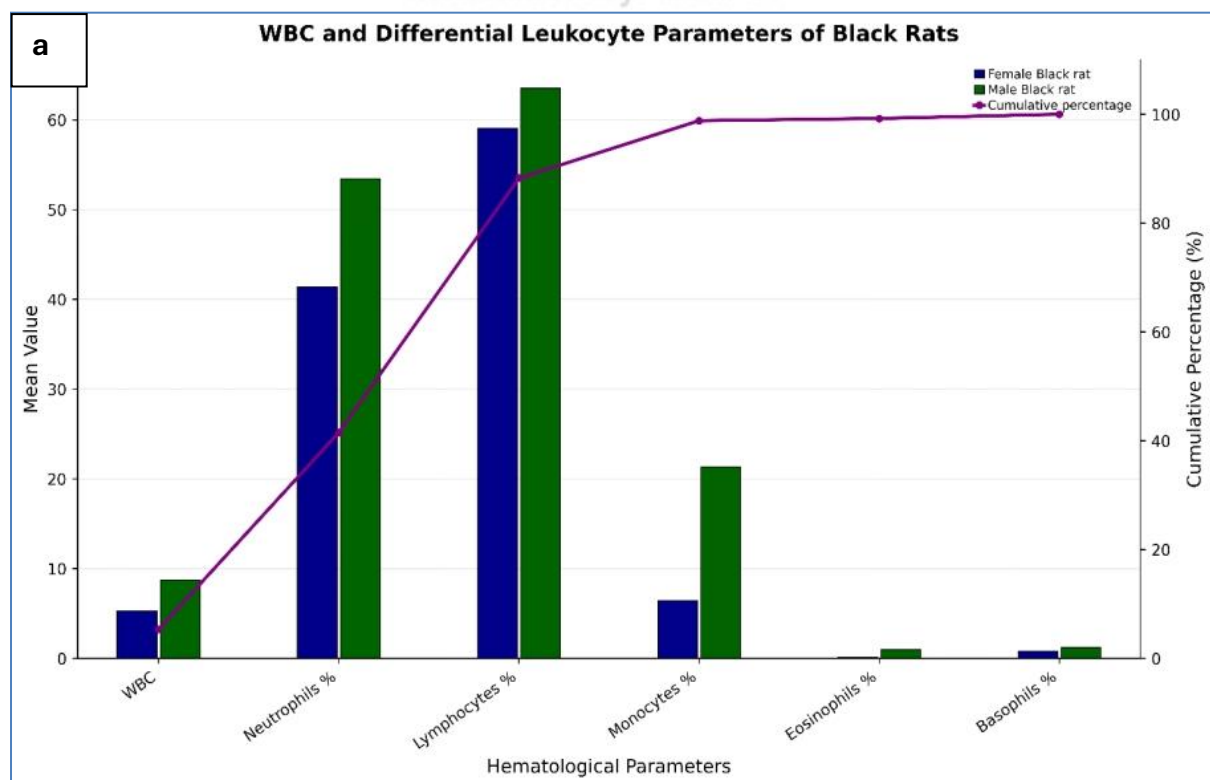
and hemoglobin level (11.6 g/dL) of male black rats were slightly higher than those of females (5.7 and 10.3 g/dL, respectively), accounting for 5% and 14% of the cumulative percentage contribution. On the other hand, females had

a higher mean MCV than males (54.8 fL and 51.8 fL, respectively) and the highest overall percentage (58% cumulative percentage). Male black rats had a higher mean corpuscular hemoglobin (MCH) than females (21.5 pg vs. 18.7 pg, respectively, which brings the total up to 74%), and female black rats had a higher mean corpuscular hemoglobin concentration (MCHC) than males (34.4% vs. 31.8%, respectively, which completes the cumulative percentage of 100%) (Figure 8b).

White Blood Cell and Differential Leukocyte Parameters of Black Rats

A comparison of the leukocyte and leukocyte differential parameters of black rats showed that the parameter overall mean values were higher in the male rats in almost all parameters. The total white blood cell count

was higher in males (8.7) than in females (5.2) and together made up 5% of the cumulative percentage distribution. The major portion of leukocytes in both sexes was composed of lymphocytes, which averaged 63.6% in males and 59.1% in females and thus brought the total percentage to 89% after neutrophils, which averaged 53.5% in males and 41.4% in females (42% cumulative percentage). There was a significant difference between the mean percentages of monocytes in males and females (males 21.2%, females 6.4%, cumulative distribution 99%). The granulocyte subpopulations were minor in both groups, with eosinophils being the highest at 1.0% for males (99.5% cumulative) and 0.1% for females (99.8% cumulative), while basophils make up 1.2% of males (99.5% cumulative) and 0.8% of females (99.8% cumulative) (Figure 8a).



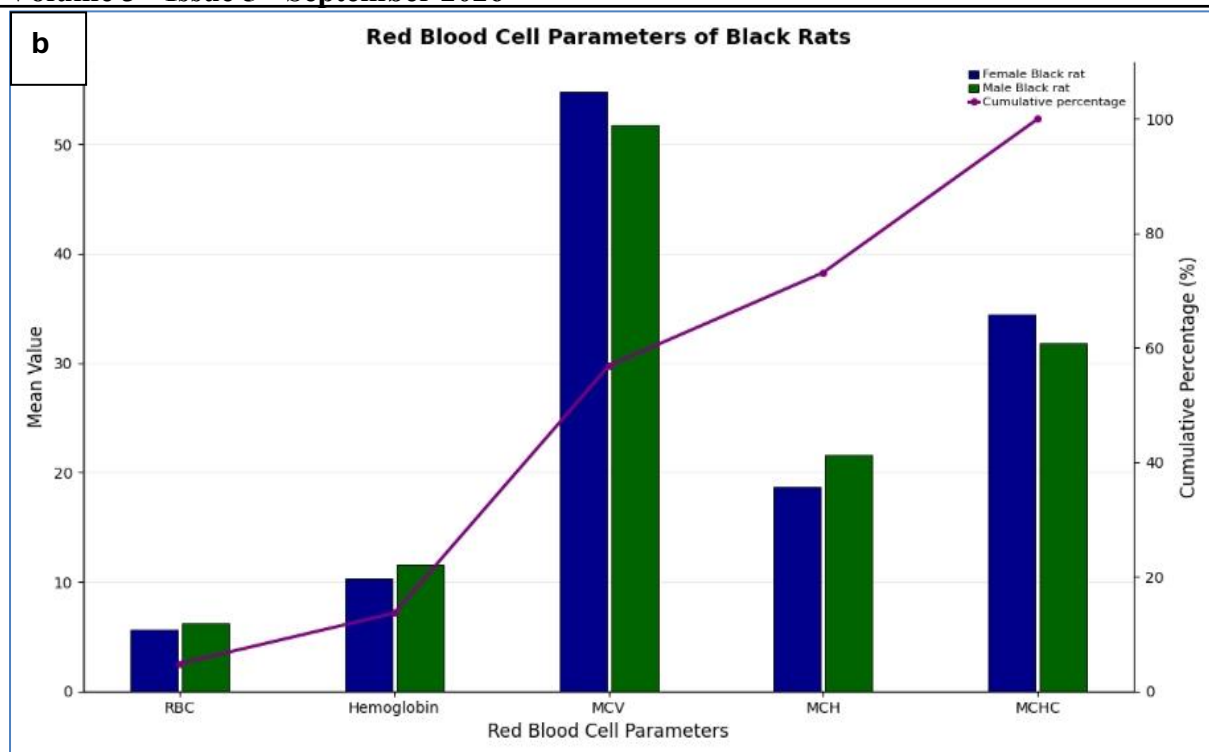


Figure (8a-8b): a) Red blood cell parameters and cumulative percentage distribution in female and male black rats b) Total white blood cell count and differential leukocyte percentages with cumulative percentage overlay in female and male black rats.

Discussion

Hematological profile of brown rats

The present study was conducted to compare the differences in haematological parameters between female and male brown rats (*Rattus norvegicus*) with leukocytic, erythrocytic and platelet parameters. Hematological parameters are significant markers of rodents' physiological state, immune system, nutritional status and health status. These parameters can vary due to species, sex, age, reproductive state, nutrition, exposure to the environment, stress and disease (Aydemir et al., 2023; Kusmeirczyk et al., 2020). For the present study, hematological parameters did not significantly differ between females and males, and both sexes were physiologically and hematologically quite similar. The difference in erythrocyte hemoglobin content was, however, statistically significant for MCH and may reflect a sex-related difference.

White blood cell count

The number of white blood cells was nearly equal in female and male brown rats. Females

had a mean WBC count of $8.61 \pm 2.00 \times 10^3 \mu\text{L}^{-1}$, whereas males had a mean WBC count of $8.60 \pm 0.97 \times 10^3 \mu\text{L}^{-1}$, with no significant difference ($p = 0.9$). The nearly equal values indicate that there was little difference in total circulating leukocytes as a result of sex in the rats under study.

White blood cell (WBC) counts are an important part of the immune system and can fluctuate with exposure to environmental contaminants, physiological stress, nutritional status, inflammation and infection (Saleem et al., 2025; Mahraan et al., 2025). This similarity between the two sexes may thus mean that broad environmental and physiological conditions existed in both sexes. Siegel and Walton (2020) and Hawkey (2017) stated that haematological parameters can be useful for obtaining information on the health conditions of wild and laboratory rodents, especially when animals are subjected to variations in their environment.

This is also similar to the overall principle that hematological parameters are not always solely sex dependent, as many environmental and

biological factors are at play. Leucocyte distribution and production can be affected by age, reproductive status, food availability, pathogen exposure, and physiological stress (Chmielewski & Strzelec, 2018; Becker et al., 2018). Thus, sex did not appear to be a major factor influencing total leukocytes in the present population, as there was no significant difference in WBC count.

Differential leukocyte profile

There were no statistically significant differences between female and male brown rats in the differential leukocyte count. Neutrophils were $45.4 \pm 20.5\%$ in females and $48.7 \pm 3.6\%$ in males ($p = 0.5$), while lymphocytes were $55.1 \pm 4.9\%$ and $53.5 \pm 4.7\%$, respectively ($p = 0.8$). Similarly, monocytes were $32.3 \pm 6.6\%$ in females and $37.5 \pm 8.8\%$ in males ($p = 0.6$). Eosinophils also showed very similar values between females ($0.55 \pm 0.23\%$) and males ($0.48 \pm 0.13\%$, $p = 0.8$).

Overall, there were no significant differences among these parameters, indicating that the overall immune cell profile was fairly consistent across sexes. Neutrophils are key in the primary immune response to tissue damage and infection, while lymphocytes are involved in adaptive immune responses. Monocytes are involved in inflammatory responses and tissue defense, and eosinophils are associated with parasitic and allergic responses. There is therefore a potential for significant variation in these populations, which may reflect variation in immune stimulation or the environment. The present findings, however, do not support a large sex-associated difference in immune cell distribution (Rosales, 2020; Li et al., 2019; Jaillon et al., 2013).

The leukocyte profile is influenced by the environment in rodents. Pollutants, changes in food, temperature variations and pathogen pressures can alter immune responses (de Sousa Fernandes et al., 2022; Becker et al., 2020). Rodents are especially suitable for the measurement of such effects, as they are adapted to a wide variety of habitats and can be subject to a high level of environmental variability (Buchholz et al., 2019). The similar leukocyte profile found in the present study

may thus suggest relatively similar environmental exposure for male and female brown rats.

Monocytes, eosinophils and basophils

A slight sex difference was observed, with monocytes being slightly elevated in male rats compared to female rats, but this difference was not significant. Male rats recorded $37.5 \pm 8.8\%$, compared with $32.3 \pm 6.6\%$ in females. The numerical difference should therefore be treated with caution since the p value (0.6) suggests that there is not enough evidence to conclude that there is a difference between the sexes.

Similarly, there was no significant difference between the number of eosinophils in males and females. Females had $0.55 \pm 0.23\%$, whereas males had $0.48 \pm 0.13\%$. There was no significant difference between the sexes ($p = 0.8$), which suggests that the eosinophilia was generally similar. Basophils were also similar, with a value of $\sim 1.2 \pm 0.2\%$ in females and 1.1% in males ($p = 0.8$). Overall, these results indicated that the more subtle differences in the number of each type of leukocyte in individual samples were not enough to show meaningful differences between the sexes.

Interpretation of individual leukocyte percentages should be based on clinical observations, other hematological indices and leukocyte levels that can vary according to environmental factors, stress and parasitic exposure (McClure, 1999; Pilny, 2008). The present results are therefore interpreted more correctly as evidence for a generally similar immune profile but not as evidence of complete physiological similarity between the sexes.

RBC and hemoglobin

There was some numerical difference in the erythrocytic parameters of male and female brown rats, but most were not statistically significant. Female rats had an RBC count of $5.60 \pm 0.29 \times 10^6 \mu\text{L}^{-1}$, whereas males had $6.25 \pm 0.45 \times 10^6 \mu\text{L}^{-1}$. The mean RBC count was found to be higher in males, but this difference was not statistically significant ($p = 0.2$). In the same way, the hemoglobin concentration was higher among males than females (11.53 ± 0.49

g/dL vs 10.30 ± 0.62 g/dL), but the difference was not significant ($p = 0.1$).

The male group had higher RBC and hemoglobin levels, but the differences were not significant; therefore, there is inadequate evidence of a sex difference in these parameters in this population. Physiological activity, nutritional status, age, environmental factors, and the availability of oxygen affect the concentration of both red blood cells and hemoglobin, which play a key role in oxygen transport (Helman & Rubenstein, 1975; Kanas et al., 2016). Therefore, the reported numerical differences may be due to individual variation in the normal order of nature and not to sexual dimorphism.

The hemoglobin levels of males and females are also relatively similar, suggesting that both sexes had a relatively similar oxygen-carrying capacity. It has been found that hematological parameters can be significantly influenced by environmental and physiological conditions in previous studies conducted in rodents (Arias-Reyes et al., 2021; Amriani et al., 2026). The present findings therefore should be considered in the context of the ecology and physiology of the brown-rat population examined.

Mean corpuscular volume

The MCV was slightly higher in females than in males. Female rats showed an MCV of $54.8 \pm 2.3 \mu\text{m}^3$, whereas males showed $51.8 \pm 2.8 \mu\text{m}^3$. This difference was not statistically significant, however ($p = 0.4$). The mean corpuscular volume (MCV) is an average size of individual erythrocytes, and it can be used to describe the size of circulating erythrocytes.

The numerical RBC and hemoglobin were lower in females, and the MCV was slightly higher, which could suggest a difference in RBC characteristics between males and females. However, due to the lack of significance, it can be said that there is no significant difference between female and male brown rats in terms of erythrocyte size. Nutritional status, age, physiological state and erythropoietic activity may affect the variation in erythrocyte indices (Grau et al., 2018).

Mean corpuscular haemoglobin

There were no significant differences among the different erythrocytic parameters, with the exception of MCH, which was significantly different in the two sexes of brown rats. The MCH value for female brown rats was 18.62 ± 0.76 pg, and that for males was significantly higher at 21.60 ± 1.10 pg, $p = 0.02$.

MCH is the mean cell hemoglobin, which is the average hemoglobin found on the average red blood cell. The higher value in males thus suggests that on average, the individual erythrocytes of male brown rats had more hemoglobin than those of female brown rats. This difference was observed, and there was no significant difference in the RBC count, hemoglobin concentration or MCV. Even when the parameters of the overall erythrocytic profile are quite similar, the presence of such a finding indicates that there can be differences in some of the erythrocyte parameters between males and females.

The observed sex difference in MCH could be related to the physiological and hormonal differences between males and females, but these differences were not measured in the present study. (Mauvais-Jarvis, 2024; Maggi & Della Torre, 2018). The data from the present paper, however, do not allow one to draw a conclusion as to the exact mechanism that brought about the increase in the MCH in males. It would be interesting to expand on this study to include the factors of age, reproductive status, body weight, hormonal status and nutritional condition.

The MCHC was 34.45 ± 1.00 g/dL in females and approximately 31.80 g/dL in males. However, the difference was not statistically significant ($p = 0.1$). MCHC gives information on the concentration of hemoglobin inside a blood cell, and in combination with MCV and MCH, it will provide a more complete picture of the characteristics of the blood cell.

The significant difference noted in MCH was not significant when applied to the total hemoglobin level of erythrocytes, as reflected in the lack of a significant difference in MCHC. This demonstrates that individual haematologic parameters should not be used alone but in conjunction with other parameters and that they may not be the most important parameters. Similar approaches have been

recommended for studies of the hematological system in rodents, as a number of indices may be influenced by environmental, physiological, and nutritional factors (Davis et al., 2008; Malcovati et al., 2003).

Platelet count

There were also similar numbers of platelets in both sexes. Females had $437 \pm 48 \times 10^3 \mu\text{L}^{-1}$, whereas males had $479 \pm 61 \times 10^3 \mu\text{L}^{-1}$. The mean platelet count was not significantly different between male and female rats ($p = 0.5$). Platelets are involved in crucial functions, such as blood clotting and the maintenance of vascular integrity, and important fluctuations in platelet counts and properties might occur in the presence of pathological events, inflammation or physiological stress (Tian et al., 2025; Eisinger et al., 2018).

Male and female brown rats did not differ significantly with regard to hemostatic function involving platelets, and the platelet function was basically similar in both groups of brown rats. The range of numbers may be due to the natural range of individual differences. Environmental factors, nutritional status, age and health conditions may influence hematological parameters. These factors should be considered when interpreting platelet counts in laboratory rodents, especially those that are not domesticated but are wild or semiwild (Santos et al., 2016).

Variation in morphometric features according to sex

This project found sex differences in several of the biometric quantities of *Rattus norvegicus* and *Rattus rattus*. The males of *R. norvegicus* on average were heavier than females (126 g v 106 g), and there were also differences in head-body length, body width, body girth and tail length. Some physiological and physiological needs differences between men and women may be related to similar sex differences in body characteristics (Helman & Rubenstein, 1975; Maggi & Della Torre, 2018; Mauvais-Jarvis, 2024). With males generally having greater values than females for a number of body dimensions, a similar pattern was observed in *R. rattus*. However, the observed variation could have been due to variation in

age, nutritional status, reproductive state, habitat and environmental experiences of the captured animals and thus may not be attributed to genetic variation (Buchholz et al. 2019; Becker et al. 2020). Thus, these results were not intended as evidence of differential growth but rather as sex differences in biometric traits. The numerical values of males were often higher for a number of morphometric features, but it is advisable to use appropriate statistical tests to determine the significance of the differences. The need for larger sample sizes and controlled age and reproductive conditions is suggested for further investigations.

Conclusion

The present study demonstrates that wild brown and black rats from Sargodha exhibit measurable sex-associated variation in morphometric characteristics, whereas their overall hematological profiles remain comparatively similar between males and females. Males generally showed greater body weight and several biometric dimensions, suggesting observable sexual variation in somatic traits. Most CBC parameters did not differ significantly between sexes, indicating broadly comparable hematological status; however, MCH was significantly higher in male brown rats, representing a specific sex-associated difference in erythrocyte characteristics. At the interspecific level, the monocyte percentage differed significantly between brown and black rats, whereas most other hematological indices were comparable. Overall, the findings suggest that sex alone may not be a dominant determinant of the hematological profile in these wild rodent populations, while morphometric variation appears more pronounced. Because the animals were collected from natural environments and age, reproductive status, nutritional condition, and hormonal profiles were not controlled or measured, the observed differences should be interpreted as sex-associated rather than definitive evidence of hormonal regulation. Future studies incorporating larger sample sizes, age stratification, reproductive status, hormonal measurements, and controlled environmental

variables are recommended to clarify the biological mechanisms underlying sex-specific variation in wild rodents.

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