Physiological and hematological reference values of wild caucasian squirrels (*Sciurus anomalus pallescens*) in northern Iraq

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**Abstract**

Wild Caucasian squirrels are commonly seen in the mountain forests of the northern Iraq and are sometimes kept as pets. However, no hematological or physiological reference values are set for these animals, even though they are often taken to private veterinary hospitals in the region. So, setting these values is necessary. A selection of hematological and physiological parameters is measured in 24 wild Caucasian squirrels (20 males and four females) in the mountain areas of Pshdar district, Sulaymaniyah, north of Iraq. The hematological parameters included the leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and examination of blood smears stained with Giemsa. The vital signs included the heart rate, respiratory rate, oxygen saturation (SpO₂), and rectal temperature. The physiological parameters were scored, and blood was taken from the animals without being sedated or anesthetized by immobilizing them using a specially designed jacket. Most values were as expected compared to other rodent species, and no significant differences were observed between the sexes. All squirrels are successfully rehabilitated and released after the experiments are conducted.

**Keywords:** Blood parameters, Caucasian squirrel, Physiological parameters, Reference values

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**Introduction**

There are more than 365 species of squirrels worldwide, belonging to seven families. The three most prevalent types are the ground squirrel, flying squirrel, and tree squirrel families. Among them, the Caucasian squirrel (1) is a member of the tree squirrel family, Sciuridae (2). It is a medium-sized rodent with a chestnut grey to grizzled buff dorsum, buff eye rings, and chestnut to buff-yellow ventral fins. It is one of 28 *Sciurus* species in Middle Eastern and southwestern Asian forests (3) and is subdivided into *S. anomalus pallescens*, *S. anomalus anomalus*, and *S. anomalus syriacus*. *S. anomalus pallescens* is found in northern Iraq and the Zagros Mountains in western Iran (3). *S. anomalus anomalus* is distributed in the Caucasus Mountains, including Turkey, Azerbaijan, Georgia, Armenia, and Greece, while *S. anomalus syriacus* is distributed in the eastern Mediterranean, including Syria, Jordan, Lebanon, and Palestine (3). The pet industry is booming due to globalization and the trade market business (4). Increased ownership of exotic and non-domesticated animals resulted in an increase in non-native pets, which resulted in the expansion of invasive alien species in the natural habitat (5). On the other hand, this phenomenon harmed biodiversity, human health, and the economy. Squirrels are allochthonous non-native pets introduced from the wild into human habitats. The main known species of the Sciuridae family in the Middle East is the Caucasian squirrel. There are more of these rodents visiting veterinary clinics now since people are starting to keep them as pets at home (1,6). These rodents are a vast and charismatic category of mammal species (5) whose attraction to people makes them...
commonly present in private houses and zoos (5). The Caucasian squirrel has become one of the most common exotic animals as companion pets in northern Iraq. So, increasing health status has become an essential tool in health protection and a unique challenge for squirrel species (7). It is crucial to ensure that animals have a high quality of life. For many terrestrial animals, welfare evaluation is relatively well established. In both human and animal medicine, physical status average value measurement and blood examination are carried out for various reasons, including as a screening procedure to evaluate general health or diagnose disease and as a tool for patient monitoring to ascertain how they are doing with specific disease cases. The vital organs’ function assessment should be undertaken without a sedative or anesthetic agent since anesthesia significantly impacts the blood and physiological parameters in lab animals and exotic species (8,9). However, determining the average physical status values is challenging in wild exotic animals, such as squirrels, without administering a sedative or anesthetic agent, as these animals are easily stimulated, resulting in abnormal heart and respiratory rates. Therefore, establishing physiological and hematologic parameters without sedating or anesthetizing the animal is essential for evaluating the health status. Two comprehensive studies addressed several average hematolgy values in Caucasian squirrels (2,10). However, the examinations were under anesthesia or sedation, which might give false data. Also, the data published in the previous studies might not be used as a standard for all squirrels since these values might change according to the wild animals' environment.

The present study aimed to measure the normal physiological and total hematologic values of Caucasian squirrels without anesthetizing or sedating them and to establish the physiological and hematologic parameters and morphological characteristics of the blood cells of Caucasian squirrels in northern Iraq.

Materials and methods

Animals

Twenty-four healthy Caucasian squirrels (Sciurus anomalus pallescens) were trapped in their rural natural habitats, the mountain forests of Pshdar countryside in the north of Iraq (KRG). Pshdar forest and mountain are situated in the upper northeastern part of Iraq (36.18° N and 45.12° E), ranging in altitude from 1,112 m to 3,648 m, and are 128 km away from Sulaimaniyah. The natural diet of this squirrel species is oak (Quercus calliprinos). The trapping, handling, and management procedures of the squirrels were under the permission of the Ethics Committee at the College of Veterinary Medicine, University of Sulaimani (Approval no. AUP-2021-19). After being collected, animals were transported into veterinary care to examine for abnormalities and infectious diseases. These include the overall health status, wounds and scars, external parasites, and skin condition. Then, the healthy squirrels were housed at the animal house of the Veterinary Teaching Hospital, College of Veterinary Medicine, University of Sulaimani. The housing procedure lasted approximately two weeks for adaptation to the new diet and environment. The squirrels were housed in a special design wooden atraumatic cage (3.5 × 1.5 × 2.0 m³). The cage was divided into three equal compartments, and the ground in each compartment was raised with wood flack and tree leaves to make the condition similar to a natural habitat. Animals were nested in different-sized handmade clay pots (20-50 cm) as a natural nest (11), and embedded with cotton, leaves, and grass material.

The squirrels’ diets and water were provided ad libitum. The food was a balanced diet from a mixture of walnut, oak, hazelnut, pinenut, peanut, pumpkin seed, sunflower seed, almond, fiber source (wheat bran), minerals, and vitamins to meet their nutritional requirements.

The squirrels included in this study were 20 adult males and four females ensnared in the mountain areas of Pshdar. Age was determined depending on the genitals and nipple color, tail molt pattern and pigmentation, hair coverage, color and dimension of the vulva, and position of the testicles. During the experimental study, one female squirrel (a companion pet) was introduced to the cage to decrease fear and animal anxiety from handling. Because the Caucasian squirrel is a naturally diurnal species, sunlight was used as the natural source of light and temperature.

A routine daily check of the health status of squirrels was performed, and the cage was cleaned three times a week. Squirrels were placed in individual clear plastic animal containers (30×19×20 cm³) and moved from the housing location to the designated examination room (approximately 25 °C).

Restraining procedures

Manual handling, controlling, and immobilizing exotic wild animals encounter extreme difficulties because non-companion squirrels are fractious, with powerful jaws and agile feet, often requiring chemical restraint and sedation procedures, while collecting blood samples before producers require physical restraint only. As a result, we used a jacket to control and restrain animals.

Physiological parameters

Vital signs were obtained from individuals at the resting stage, including individual rectal temperature (RT), respiratory rate (RR), oxygen saturation (SpO₂), and heart rate (HR). The measurements were taken on resting squirrels without unnecessary excitements to provide accurate values. Rectal temperature was measured by inserting the tip of a thermometer probe 1 cm into the rectum and keeping in touch with the rectal wall until it gives a maximum degree of temperature (Figure 1). The rectal temperature value shown on the screen display monitoring device was recorded as the
rectal temperature. Respiratory rate was measured by counting the number of exhalations per minute while monitoring the airflow after placing a blackbird’s feather against the nostrils of the squirrel. Simultaneously, the device’s respiration moment was counted to double-check the collected values. Heart rate was measured by counting the duration of R-R wave intervals of ECG papers after placing ECG leads on animals’ limbs valued as beats/minute. The heart rate was calculated as 3000 ± numbers of small squares between R-R intervals.

Oxygen saturation (SpO₂) was recorded after securing the pulse oximetry clump on the tip of three fingers of squirrels. Parameters were continuously monitored with a monitoring device (Veterinary Monitor, type KM-12, Keebomed, USA), shown in figure 1.

![Figure 1](image1.jpg)

**Figure 1:** Measuring the vital signs of a squirrel after immobilization with a jacket (left) and collecting 0.5 mL of blood from the cephalic vein of a restrained squirrel with a jacket without sedation or anesthesia (right).

### Hematology

The animal’s middle arm was prepped by clipping and shaving it, then sterilizing it with 70% alcohol. The cephalic vein was occluded with digital pressure by an assistant to visualize the vein. About 0.5 mL of blood was drawn from one of the collateral cephalic veins using a 25-gauge needle and a 1 mL syringe. The pressure was applied with gauze at the needle exit site for hemostasis. Blood samples were taken in a heparinized pediatric tube (0.5 mL with EDTA) to preserve a small blood volume for longer (12,13). The blood sample was identified using dyes that equal numbers (each dye equals a number), and the blood sample was then ready for complete blood counting (CBC) with an automatic hematology analyzer device (HTI MicroCC-20Plus, MCC-2000-V0, high Technology Inc. Walpole, USA). The following blood parameters were measured: total leukocytes, lymphocytes, granulocytes, erythrocytes, hemoglobin (HB), hematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (14,15). Morphological examination of differential leukocytes was obtained with Wright-Giemsa stain of blood smear under 1000× magnification power.

### Results

The study included 24 squirrels (20 males and four females), and the data of both sexes were combined since the researchers could not obtain equal numbers from both sexes. After physical examination, all squirrels appeared healthy, depending on vital signs and their body condition. The squirrel’s weights ranged between 255 g and 410 g, averaging 280.0±9.1 g. The average heart and respiratory rates were 342.9 and 106.3, respectively, while the average rectal temperature was 38.9 ºC, and the SpO₂ was 97.3% (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mean±SEM)</th>
<th>Median</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (breaths/min)</td>
<td>342.9±8.6</td>
<td>301</td>
<td>286-423</td>
</tr>
<tr>
<td>Respiratory rate (beats/min)</td>
<td>106.3±2.1</td>
<td>107</td>
<td>85-125</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.9±0.2</td>
<td>39.1</td>
<td>38.3-39.9</td>
</tr>
<tr>
<td>Oxygen Saturation (%)</td>
<td>97.3±0.2</td>
<td>97</td>
<td>95-99</td>
</tr>
</tbody>
</table>

Erythrocyte examination showed polychromatic and Howell-Jolly bodies (HJJs). Polychromatic cells are realized as multicolored erythrocytes noted in 70.8% (17/24) of the squirrels and were mildly and moderately distributed in 45.8% (11/24) and 25.0% (6/24) of the animals. HJJs were recognized as dark blue to purple inclusion bodies peripheral to the erythrocyte in approximately 41.7% (10/24) of the squirrels. The microscopic examination of leukocytes revealed different morphological characteristics of neutrophils, basophils, eosinophils, kidney-shaped lymphocytes, and large granular monocytes (Figure 2).

Platelets appeared light to dark purple smaller than erythrocytes. Based on distribution, 32.0% of the platelets appeared single, and 68.0% appeared as large clumps among erythrocytes and leukocytes. The average erythrocyte count was 8.4 × 10⁹/µL, and the hemoglobin content was 13.3 g/dL. The hematocrit was 37.6%, with an MCV of 44.5 fl. Also, the total leukocyte count was 4.6 × 10⁹/µL, with neutrophils accounting for 62.0% of the cells, while the lymphocytes constituted 28.9% and the other cell types added up to 9.0% (Table 2).
Discussion

Measuring the physiological parameters of wild animals has always been challenging since exotic species will become stressed in their new environments. Hence, reducing the stress factors affecting the data's accuracy is crucial when a wild species' physiological parameters are scored. During the current study, any source of stress was eliminated, including olfactory, auditory, and visual. We also considered animal restraint times during the physical examinations and blood sample collections. The body releases adrenaline and cortisol (stress hormone) during stress and physical handling, and adrenaline causes an increase in heart and respiratory rates (13,14). Also, a recent study evaluated glucocorticoids in the feces of red squirrels and showed that physical handling and keeping squirrels in captivity increased stress hormone levels (15).

To the researchers' knowledge, this study describes the average physiological parameter values (HR, RR, SpO₂, RT) in Caucasian squirrels without being sedated or anesthetized for the first time. Using these agents probably causes inaccurate results, and previous studies used sedatives or anesthetics before collecting the physiological data. Squirrels have been the subject of several studies to assess their physiological condition when under general anesthesia, but the process significantly affects the physiological values regardless of the species.

Table 2: Total hematological parameters of wild caucasian squirrels in the current study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERY (×10⁶/µL)</td>
<td>8.4±0.5</td>
<td>6.5-10.4</td>
</tr>
<tr>
<td>LEU (×10⁹/µL)</td>
<td>4.6±0.8</td>
<td>2.6-7.9</td>
</tr>
<tr>
<td>HB (g/DL)</td>
<td>13.3±0.6</td>
<td>10.2-15.6</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>37.6±2.4</td>
<td>26.2-44.0</td>
</tr>
<tr>
<td>PLT (×10⁹/µL)</td>
<td>767.9±43.8</td>
<td>599.0-959.0</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>44.5±1.7</td>
<td>35.4-52.8</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>15.8±0.4</td>
<td>14.4-17.5</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>35.9±1.6</td>
<td>33.1-46.6</td>
</tr>
<tr>
<td>NEU %</td>
<td>62.0±2.8</td>
<td>34.6-79.7</td>
</tr>
<tr>
<td>LYM %</td>
<td>28.9±2.8</td>
<td>5.0-56.4</td>
</tr>
<tr>
<td>MID %</td>
<td>9.0±0.6</td>
<td>1.8-15.3</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>15.5±0.8</td>
<td>13.0-20.6</td>
</tr>
<tr>
<td>RDW-SD</td>
<td>32.9±1.4</td>
<td>29.2-41.9</td>
</tr>
<tr>
<td>MPV</td>
<td>6.3±0.2</td>
<td>5.4-6.8</td>
</tr>
<tr>
<td>PDW</td>
<td>8.2±0.3</td>
<td>6.4-9.2</td>
</tr>
<tr>
<td>PCT</td>
<td>0.5±0.1</td>
<td>0.2-0.7</td>
</tr>
<tr>
<td>P-LCR</td>
<td>8.8±1.7</td>
<td>1.8-15.3</td>
</tr>
</tbody>
</table>

ERY = erythrocytes, HB = hemoglobin, HCT = hematocrit, LYM = lymphocyte, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, LEU = leukocytes, MCV = mean corpuscular volume, MID = basophils, eosinophils and monocytes combined, MPV = mean platelet volume, NEU = neutrophils, PCT = plateletcrit, PDW = platelet distribution width, P-LCR = Platelet-large cell ratio, PLT = platelet, RDW-CV = reticulocyte distribution width coefficient of variation, RDW-SD = reticulocyte distribution width standard deviation, SEM = standard error of the mean.

A recent study calculated the basal value of the heart rate of gray squirrels, which was lower than our results on Caucasian squirrels and non-hibernating tree squirrels (red squirrels and gray squirrels). The larger tree squirrels have larger bodies and lower heart rates since there is a link between resting heart rate and total body size (16).

Red squirrels and Siberian chipmunks with comparable body sizes show similar HR results, but their heart rate is slower than ground squirrels (Richardson's ground squirrel and black-tailed prairie dog). These species hibernate, which makes them physiologically unstable and subject to fluctuating physiological and metabolic conditions. Hibernation causes various physiological changes that help animals endure seasons when resources are scarce. Torpor, which lasts for many days, is characterized by severe decreases in body temperature (down to -3°C in Arctic ground squirrels), metabolic rate (2-4% of resting metabolic rate), HR, and RR (17).

While showing a negligible difference in correlation with other hibernating squirrels, the respiratory rate data seemed relevant to the red squirrel. One study on the gray squirrel's core body temperature was unrelated to our findings. As a
physiological adaptation to ambient temperature, the temperature of Sciuridae species varies with the season and decreases during periods of food deprivation. Experiments on the gray squirrel revealed oxygen saturation (SpO₂) values similar to our findings (18). The high percentage of polychromasia and HJBs in erythrocytes in our squirrels followed the Indian squirrel (Five-striped palm squirrels) (19) and other rodents such as mice (20).

The total leukocyte count of our squirrel is similar to a previously reported finding in Zagros Mountain (high altitude) squirrels (2) but was higher than the squirrels of the Tehran Zoo (low altitude) (10). Based on the hematological study on squirrels, higher altitude areas where animals live lead to higher production of erythrocytes due to a greater demand for oxygen (21). Nonetheless, a variety of nutritional factors and degrees of animal stressors, and various homeostatic mechanisms affect erythrocyte production (2). A similar profile is present in tree squirrels, whereas the erythrocyte concentrations were higher than in ground squirrels and lower than in chipmunks. This agreement with habitat plays a significant role in the hematological profile (21,22). Several changes in blood composition occur before entering torpor or during torpor itself in hibernating squirrels, and these changes may help torpid animals survive low levels of oxygen and substrate delivery to the brain. Blood volume is lowered during hibernation, and plasma erythrocyte and leukocyte concentrations are also decreased, which may partially explain how hibernating ground squirrels can endure significantly decreased brain oxygen and substrate supply. The persistence of circulating leukocytes might trigger an inflammatory cascade that results in cerebral ischemia, similar to what is seen after a stroke or severe head injury if their concentrations are not lowered (23).

The average HCT value of our adult animals was different from Persian squirrels (same species), tree squirrels, and chipmunks, but it was close to the HCT of ground squirrels. Several factors might decrease HCT levels, including age, as older individuals have lower hematocrit values (24). The average platelet counting of our squirrels was relatively higher than the captive Caucasian squirrel, but a similar range was found with the five-striped squirrels. Small mammals frequently exhibit thrombocytopenia in lab tests, although this finding is sometimes erroneous due to problems with blood collection and platelet clumping (25). The average leukocyte count was close to other studies' results, with similar results of total counting and percentage of neutrophils and mid cells (basophils, eosinophils, and monocyte), as well as lymphocytes. Moreover, our study is the first to describe some hematological parameters in Caucasian squirrels, such as the RDW-CV, RDW-SD, MPV, PDW, PCT, and P-LCR. The MPV, PDW-SD, and PDW were lower than the result of eastern gray squirrels. Several factors are associated with hematological parameter differences, including age, environmental conditions, diet, and sex (26). Specific reference values in wild species may be influenced by stress, handling, and restraint method with the compact effect of anesthesia (27).

Conclusion

This study fulfills two goals. First, it provides the common hematological reference values for wild Caucasian squirrels without potential adverse effects associated with anesthesia. These values may be a standard profile for healthy Caucasian squirrels in captivity and the wildlife in the north of Iraq. Second, it also provides the average vital parameters as a reference interval for diagnosing a clinical disease of squirrels and indicates how those values differ according to geographical locations and species variation in parameters with other species of Sciuridae.

Acknowledgment

The University of Sulaimani financially supported this study.

Conflict of interest

The authors declare that they have no conflict of interest.

References

The document appears to be a scientific paper discussing various aspects of animal physiology and behavior, particularly focusing on the hibernation process in different species. It includes references to various species of squirrels and other small mammals, suggesting a study on their physiological and molecular responses to depressed metabolism and low temperature.

The text mentions specific studies and experiments, such as the effect of different substances on heart rate variability and measures of body temperature, and the coordination of multiple physiological and molecular events during preparation for and entry into torpor. It also references studies on the behavioral and physiological effects of captivity, sex, and season on different species, including the eastern spotted skunk and the racercoon.

Overall, the paper seems to be a comprehensive review of the topic, incorporating a variety of research findings from different studies.

References are likely included in the text, providing further details on the studies and experiments discussed. The document appears to be a valuable resource for anyone interested in the field of animal physiology and behavior.