Histological Description and Histometric Assessment of the Peripheral Blood Cells in the Saw-Scaled Viper (*Echis carinatus sochureki*)

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**Abstract:** A histological and histometrical study of peripheral blood cells of saw-scaled viper (*Echis carinatus sochureki*) from southwestern Iran was performed. Blood smears were prepared immediately, air-dried and differential leukocytes count were done. Also, histometric measurements of the various blood cells were carried out. The characteristic shape of the saw-scaled viper erythrocytes was oval and enucleated. Overall, five types of the leukocytes include heterophils, eosinophils, basophils, lymphocytes and monocytes were observed in the peripheral blood smears. The results of the present study are useful for the scientists involved in snake conservation around the world and emphasize the usefulness of the microscopic studies in viper conservation.

**Key words:** Histology • Histometry • Blood cells • *Echis carinatus sochureki*

**INTRODUCTION**

The snake fauna of Ilam province, southwestern Iran has the most biodiversity due to variety of climatic regimes of this province and also diverse of geographic condition such as mountains, alluvial fans and sandy area and gypsum hills [1]. In addition, it has been reported that of 87 species and subspecies of snakes recorded in Iran [2], approximately 31% occur in Ilam province [2, 3].

Since reptiles are very sensitive to changes of habitat checking their blood parameters may guide the evaluation of physiological and health conditions and may be used as a good indicator in determining environmental conditions [4-7]. In addition, due to need for conservation actions [8], diagnostic evaluation of reptiles [9], economic importance of species and comparative study [10]; a thorough knowledge of reptilian blood histology is becoming more imperative.

*Echis carinatus sochureki* is a venomous viper species found in parts of the world including western Iran. At the first time, taxonomy, morphology, distribution and habitat of the saw-scaled viper from Ilam province were described by Latifi [11] but there is not a comprehensive study on the blood cell morphologies of this species. So, the present work was performed to obtain the histological description and histometric assessment of the peripheral blood cells in the saw-scaled viper (*Echis carinatus sochureki*).

**MATERIALS AND METHODS**

Study area was Ilam province which is located in the western and southwestern regions of the Iranian Plateau between 31°58' and 34°15' N and 45°24' and 48°10' E. *Echis carinatus sochureki* had been rehabilitated in the wildlife laboratory of the Para-veterinary medicine college in the Ilam University (Ilam, Iran). The snakes were identified according to Latifi [11] descriptions using morphometric measurements, coloration and pholidosis features [11]. Blood samples were collected from ventral caudal vein just before the vipers were set free, when they were clinically normal and in good physical condition.

Twenty blood smears for each snake were prepared immediately and air-dried. For differential leukocytes count the blood smears of each viper were stained with a quick Romanowsky-type stain, Diff Quick (QD), according to the manufacturer's instructions. Two hundred leukocytes were counted for each blood smear for differential relative count. In addition, for description of the microscopic characteristics of the blood cells the blood smears were soon stained with Wright's and Giemsa.
method. The stained smears were studied under a light microscope (Nikon Eclipse E800) and appropriate photographs of the blood cells were taken with a digital camera (Nikon, China) and stored.

For histometric assessment, from each blood smear, 100 erythrocytes were randomly chosen for the measurement of their lengths (L), widths (W), nuclear lengths (NL) and nuclear widths (NW). Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed from ES= LWπ/4 and NS= NLNWπ/4. Comparisons of cell and nuclear shapes were done from L/W and NL/NW ratios and that of nucleus/cytoplasm from NS/ES ratio. From the blood smears of each snake, measurements of leucocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) and thrombocytes (TL, TW) were also taken to determine their sizes. One way ANOVA test was utilized in the comparisons of the obtained data, α= 0.05 in all of the analyses.

RESULTS

The results of the leukocyte differential count from saw-scaled viper are inserted in the Table 1. Heterophils were the most numerous cells among various leukocyte cells, followed by lymphocytes. Basophils, monocytes and eosinophils were very small number in the white blood cells count.

The erythrocyte lengths, widths, sizes, L/W ratios, nuclear measurements of the erythrocytes and also nucleocytoplasmic rations (NS/ES) are given in Table 2. Erythrocytes were homogeneous in color but moderately anisocytosis. Their shape was oval and enucleated. The erythrocytes have a violet-blue ellipsoidal sometimes indented nuclei uniformly localized in a central part of the cell. On smears stained with Wright's and Giemsa method, the cytoplasm were light pale pink and the chromophilic nuclei were dark purplish blue. (Fig. 1-1).

In the present work, leukocytes of saw-scaled snakes were categorized into 5 groups; heterophils, eosinophils, basophils, lymphocytes and monocytes. Measurements on the various leukocytes and also thrombocytes in the blood smear of the saw-scaled viper are given in Table 3.

Heterophils were the largest of the leukocytes and average 11.59 µm in diameter. They were the most prevalent circulating cells and contained large numbers of irregular shape, dull eosinophilic granules. Their nuclei were round and usually placed close to the cells periphery and in the most cases, heterophils granules were not seen easily. When the nucleus was centrally located, it acquired a round form (Fig. 1-2).

Eosinophils contained numerous round and light blue granules that often occluded visualization of the nucleus. They relatively were large-sized cells (10.83 µm in diameter) with a generally eccentric, round, darkly stained nuclei. (Fig. 1-3). Basophils were very low, average 5.1 µm in diameter and were slightly smaller than Basophils were difficult to find in the peripheral blood smears of the saw-scaled viper. These cells were round and contained a violet-blue with generally eccentric nucleus. Their purplish black cytoplasmic granules masked the nuclei, so their shapes were not readily distinguishable (Fig. 1-4).

| Table 1: Mean± standard deviation and range of the leukocyte differential count values of saw-scaled viper |
|-----------------------------------------------|------------------|------------------|
| White blood cells                            | Mean ± standard deviation | Range            |
| Heterophils (%)                              | 74.89 ± 6.37     | 49.66 - 85.38    |
| Eosinophils (%)                              | 1.02 ± 0.03      | 0.4 - 1.94       |
| Basophiles (%)                               | 1.96 ± 0.09      | 0.1 - 3.98       |
| Lymphocytes (%)                              | 15.45 ± 3.92     | 12.87 - 27.14    |
| Monocytes (%)                                | 5.97 ± 0.38      | 3.69 - 8.24      |

| Table 2: Mean± standard deviation of the erythrocyte measurements and dimensions of their nuclei on blood smears of the saw-scaled viper |
|------------------------------------------------|------------------|------------------|
| Erythrocyte measurements                      | Erythrocyte nuclei dimensions |
| L(µm)                                         | W(µm)     | L/W               | ES(µm²) | NL(µm) | NW(µm) | NL/NW | NS(µm²) | NS/ES |
| 8.05 ± 0.05                                   | 5.92 ± 0.46 | 1.42 ± 0.03       | 96.9 ± 3.27 | 4.56 ± 0.02 | 3.85 ± 0.04 | 1.19 ± 0.17 | 28.82 ± 0.68 | 0.29 ± 0.02 |

| Table 3: Mean± standard deviation of the various leukocytes and thrombocytes measurements on blood smears of the saw-scaled viper |
|-----------------------------------------------|------------------|------------------|
| Leukocytes                                    | Thrombocytes     |
| Heterophil (µm)                               | Eosinophil(µm)  | Basophil (µm)    | Small lymphocyte (µm) | Large lymphocyte (µm) | Monocyte (µm) | TL (µm) | TW (µm) |
| 11.59 ± 0.12                                  | 10.83 ± 0.02    | 5.18 ± 0.04      | 6.29 ± 0.93          | 7.75 ± 0.48           | 11.42 ± 0.15  | 4.85 ± 0.08 | 4.67 ± 0.01 |
In the blood smears, both small and large lymphocytes were observed. Large lymphocytes had a relatively wider zone of cytoplasm and well-defined round purplish blue nucleus (Fig. 1-5). In small lymphocytes, the nuclei were prominently clumped chromatin and surrounded by a rim of moderate basophilic cytoplasm. On the other word, cytoplasm was pushed to a side as a small peripheral zone (Fig. 1-6).

Monocytes were large cells and had a round or amoeboid shape. Their nucleus was purple-blue, just kidney-like form or fusiform, typically eccentric location and had a chromatin pattern slightly less clumped than that of the large lymphocytes. The cytoplasm was weak to slightly basophilic and sometimes containing variably sized intracytoplasmic vacuoles (Fig. 1-7).

Thrombocytes were round cells which were observed as groups of three or more cells in the peripheral blood smears. The nuclei was generally round, quite chromaphilic and filling the whole cell. Under the Wright’s stain, these cells were similar to the small lymphocytes in size but could be differentiated by their very pale coloration cytoplasm (Fig. 1-8).

**DISCUSSION**

In this work, the leukocyte differential count from saw-scaled viper was similar to those of Turkish snakes [12] but different from the observations of Salakij et al. [13] on the water snakes (*Homalopsis buccata*). This dissimilarity in results may be originates from the existence of multi-parasitic infestations include *Hepatozoon* sp. *Trypanosome* and *Haemogregarina* sp. in that study.

Heterophils were the most numerous leukocytes in the leukocyte count of saw-scaled viper, followed by lymphocytes which are similar to that reported for Turkish vipers [12] and other reptiles include sea turtles [14].

Previous studies have demonstrated the mature erythrocytes of reptiles are permanently nucleated and blunt-ended ellipsoids [15]. Similarly, the erythrocytes identified in our study were oval and enucleated, just like those of other reptilian vertebrates and those of birds. Also, the morphologic characteristics of erythrocytes from saw-scaled viper in this study were similar to those described in Turkish *Viperidae* snakes [12]. However, there was not literature data on the saw-scaled viper erythrocyte morphology with which comparison could be made.

Regarding blood cell morphologies, it has been suggested that reptiles constitute a heterogeneous group among vertebrate [16]. We classified five types of white blood cells in the peripheral blood smears of the saw-scaled viper: heterophils, eosinophils, basophils, lymphocytes and monocytes. These results agree with the observations on some Turkish snakes [12] and of Sypek and Borysenko on various reptiles [17].

Histological description and histometric assessment of the heterophils from saw-scaled viper in the present work were similar to those reported for Turkish snakes [12] and other reptiles [14, 16, 17]. In addition, in line with these results it has been demonstrated that heterophils in the reptiles are generally round cells with eosinophilic fusiform cytoplasmic granules and clear cytoplasm [14]. Reptilian heterophils have a similar function to that performed by neutrophils in mammals [18] and neutrophils are rare in reptiles, although these cells have been
demonstrated in the tuatara (*Sphenodon punctatus*) [19]. In the present study, we did not identify neutrophils in the saw-scaled viper. These results agree with the observations of Arikan *et al.* [12] on some Turkish vipers and snakes. Morphological descriptions of the eosinophils and basophils from saw-scaled viper were similar to those reported on the Turkish vipers [12] except for their size which were smaller than Turkish snakes.

Lymphocytes from saw-scaled viper were histologically and histometrically similar to those reported for various Turkish snakes [12]. These cells were not difficult to distinguish from other peripheral blood cells by their well-defined round purplish blue nucleus as well as rim of moderator basophilic cytoplasm. However, it has been suggested that lymphocytes from other species of reptiles can be difficult to distinguish from thrombocytes [20].

Based on the results obtained during present study, monocytes were relatively large cells and had a nucleus with eccentric location as well as a slightly basophilic cytoplasm which sometimes containing variably sized intra-cytoplasmic vacuoles. Similarly, Arikan *et al.* [12] reported the same morphological features of the peripheral blood monocytes on the Turkish snakes [12].

Previous researchers has been identified azurophils as a separate cell type in the peripheral blood smears of reptiles, but in the present study we did not identify azurophils in the saw-scaled viper. Similarly, no azurophils were reported in the peripheral blood cells described from various Turkish snakes [12].

Some authors described the thrombocytes in various reptiles as elliptical to fusiform nucleated cells, with centrally positioned extremely chromophilic nuclei and typically clear cytoplasm which may contain a few azurophilic granules [14-16, 20]. Also, it has been reported that activated thrombocytes appear as clusters of cells with irregular cytoplasmic margins and vacuoles and appear devoid of cytoplasm when aggregated [15]. The present study established the presence of thrombocytes with round-shaped cells which were similar to the lymphocytes but could be differentiated by their very pale coloration cytoplasm.

Owing to the absence of previous studies on blood cells in the saw-scaled viper, the current study provides baseline data regarding morphologic classification of blood cells in these vipers. Further study will be needed to describe the ultra structural properties of the peripheral blood cells from this specie.

REFERENCES


