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National Libyan records on blood cellular elements of the Long-eared hedgehog (*Hemiechinus auritus*)

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الملخص

تعتبر السجلات الوطنية في العديد من جوانب الحياة للحيوانات البرية أمرًا حيويًا. قد تعطي هذه السجلات معرفة كافية بالمعلمات البيولوجية التي تساعد في فهم سمات رفاهية هذه الكائنات في بيئتها الطبيعية. هذه الدراسة هي محاولة لوصف بعض الجوانب المتعلقة بخلايا الدم لأحد الحيوانات البرية الليبية، وهو القنفذ طويل الأذن (*Hemiechinus auritus*). ركزة هذة الدراسة على مورفولوجيا خلايا الدم عند الحيوانات حديثة الولادة والحيوانات البالغة التي تم الحصول عليها من محيط مدينه بنغازي. تم استخدام أربعة قنافذ صغيرة حديثة الولادة و20 قنفذاً بالغاً لإعداد مسحات دموية. لم تظهر السمات الهيكلية لخلايا الدم لكلا المجموعتين من الحيوانات اختلافات بارزة عن بعضها البعض. ضهر شكل الانويه في الخلايا متعددة الانويه في شكل شريطي ومتعدد الحبيبات وقد بلغ الحد الأقصى لعدد الفصوص النووية سبعة في القنافذ المولودة حديثًا وثمانية في القنافذ البالغة وكانت خيوط الربط واضحة جدًا في الغالب. بينما كانت الانوية غير مفصصه مع ملاحظة ثلاثة فصوص نووية في الخلايا حامضيه الانويه للحيوانات لكلا المجموعتين. بينما أظهرت الخلايا القاعدية للقنافذ المولودة حديثًا نوى كبيرة غير منتظمة، في حين أن تلك الخاصة بالحيوانات البالغة قد قسمت جزئيًا الى انوية مسننة. يمكن ملاحظة الخلايا الليمفاوية الصغيرة والمتوسطة والكبيرة في مسحات الدم للحيوانات حديثي الولادة والحيوانات البالغة. ومع ذلك، كانت غالبية الخلايا الليمفاوية صغيرة الحجم. تم تسجيل نوى غير مركزية ذات شكل كلوي وشكل حدوة الحصان وشكل بيضاوي ومستدير في الخلايا وحيدات الانويه من كلا المجموعتين من الحيوانات. في حين كانت الصفائح الدموية للقنافذ المولودة حديثًا والبالغ غير ذات انويه ولها أشكال مستديرة إلى بيضاوية وكانت ذات لون مظلم في مركز الخلية بينما فاتحة اللون في محيطها وأقل كثافة. في حين ظهرت كريات الدم الحمراء قرصية الشكل وعديمة الانويه وذات مظهر ثنائي التجويف في كلتا المجموعتين للقنافذ.

الكلمات المفتاحية: القنفذ الليبي طويل الأذن، عناصر الدم الخلوية، الخلايا القاعدية، الخلايا المتعادلة، الخلايا اللمفاوية.

Abstract

This study is an attempt to describe some aspects relating to the blood cells of one of Libya's wildlife animals, the long-eared hedgehog (*Hemiechinus auritus*). The investigation focused on the morphology of the blood cells in newborn and adult animals that were obtained from the city of Benghazi. Four one-day-old hedgehoglets and 20 adult hedgehogs were utilised to prepare stained blood smears. Structural features of blood cells of both young and adult animals did not show prominent variations from each other. Neutrophils nuclei had a band and multilobulated appearance. The maximal number of nuclear lobes reached seven in the newborn hedgehogs and eight in the adult and the joining threads between the lobes were mostly very obvious. The Barr body could not be observed in neutrophils of the female animals. Non-lobulated nuclei were encountered in the eosinophils of the adult animals, whereas the nuclear trilobulation could be noticed in the eosinophils of both groups of hedgehogs. Basophils of the newborn hedgehogs showed large irregular nuclei, whereas those of the adults had partially divided indented nuclei. Small, medium and large lymphocytes could be observed in blood films of the newborn and adult animals. However, the majority of the lymphocytes were small in size. Eccentrically located nuclei with kidney-shaped, horseshoe, oval and round appearances were recorded in monocytes of both groups of animals. Platelets of the newborn and adult hedgehogs were non-nucleated and had round to oval shapes. A centrally located dark area surrounded by a less dense area was obvious. The discoid non-nucleated erythrocytes had a biconcave appearance in both groups of hedgehogs.

Keywords: Libyan Long-Eared Hedgehog *(Hemiechinus Auritus)*, Blood Cellular Elements. Basophils, Neutrophils, Eosinophils, Lymphocytes.

1. INTRODUCTION

The protection of wildlife systems, of which the human being is an integral part, is vital. Libya has an estimated 87 species of mammals and 338 species of birds [1]. The hedgehogs are perhaps the most primitive, little changed from their ancestors that lived on the African continent some 20 million years ago. They have become well adapted to the arid climates that are close more or less to those of northern Africa. Mammalian blood is a highly specialized circulating fluid tissue.

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Its several types of cellular elements are suspended in a fluid medium known as plasma. Three main types of cells are recognized in blood: red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes). These cellular elements, mostly red blood cells, constitute in volume about 45% of the whole blood volume, the other 55% of the blood volume is plasma, the blood's liquid medium that has a light yellow appearance. The proportion of blood occupied by the erythrocytes is referred to as the hematocrit [2]. The mature circulating mammalian red blood cells lack nuclei and organelles. They have a discoid shape with edges that are thicker than the central portion of the cell to the extent that mostly they appear as biconcave disks. The primary function of

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the erythrocytes is to transport oxygen from the lung to the various tissues of the body and to assist in the transport of carbon dioxide from the tissues to the lung. Oxygen transport is mainly accomplished by the hemoglobin content of the red cells. The red blood cells are marked by glycoproteins that determine the different blood types. The life span of the erythrocyte shows variation depending on the species. Human and dog red cells may survive up to 120 days, whereas the life span in sheep and cats may be 150 and 80 days, respectively [3]. A limited number of micronucleated erythrocytes could be encountered in the mammalian peripheral blood due to the ineffective function of the reticuloendothelial system to remove them from circulation. On the other hand, micronucleated erythrocytes are usually present in considerable numbers with the ordinary nucleated erythrocytes featuring avian and reptilian blood [4]. The leukocytes are part of the immune system. Besides the destruction and removal of old or aberrant cells and cellular debris, they attack infectious agents (pathogens) and foreign substances [5]. They are spherical cells and larger than erythrocytes and have a nucleus. According to their appearance in stained preparations, the shape of the nucleus and the characteristic features of the cytoplasmic granules, these cells have been given different names. Granulocytes are leucocytes containing large cytoplasmic granules that have been designated as neutrophils, basophils and eosinophils. There are two kinds of apparently agranulated leucocytes: lymphocyte and monocyte [6]. They represent the most abundant type of leucocytes in humans and form an integral part of the immune system. Together, with the eosinophils and the basophils, they form the class of polymorphonuclear cells. The term polymorphnuclear is based on the multilobulated shape of the nucleus. The lobes are linked by fine threads of chromatin. The immature neutrophil (band form) has a nonsegmented nucleus taking the shape of a horseshoe. In some of the female neutrophils, the inactive X chromosome appears as a drumstick-like appendage on one of the lobes of the nucleus [7]. Neutrophils are much more numerous than other phagocytic leucocytes, the monocytes and their derivatives the macrophages [8]. These granular white blood cells play a considerable role in the immune system. They are responsible for combating infection and parasites in vertebrates. They are also involved in control mechanisms associated with allergies and asthma. The stained small granules are concentrated within the cellular cytoplasm and the nucleus is easily visible. The nucleus usually has two lobes, bilobed nucleus [8].

The basophils contain large cytoplasmic granules which obscure the cell nucleus upon examination under the microscope. The nucleus is divided into irregular lobes, but such division is usually obscured by the overlying deeply stained granules. The cytoplasmic granules are fewer and more irregular in size and shape than the granules of the other granulocytes. As with other circulating granulocytes, basophils can be recruited out of the blood into tissue spaces when needed [9]. As part of the immune system, the agranular monocytes participate in protection against blood-borne pathogens. Monocytes circulate in the peripheral blood for about one to three days and then move into tissues throughout the body. In the tissues, monocytes differentiate into different types of macrophages at different anatomical locations They possess a large smooth nucleus, a large area of cytoplasm and many internal vesicles for processing foreign phagocytized material. They are usually identified in stained smears by their large oval, horseshoe or kidney-shaped nucleus and are generally eccentrically placed [9]. Lymphocytes play an important and integral role in the vertebrate's immune system. There are two categories of lymphocytes, namely the large lymphocytes (also called granular large lymphocytes) and the small lymphocytes. Their nucleus is spherical, sometimes with an indentation. The three major types of lymphocytes are the T- cells, B- cells and the natural killer (NK) cells. Their life span extends from weeks to several years, which is very long compared to other leucocytes. The memory lymphocytes may live for the lifetime of the organism [10]. Sex influence has been reported in some aspects of the hedgehog's lymphocytes. The mean lymphocytic cellular volume and lymphocytes count of the female hedgehogs were slightly but significantly higher than those of the male. Abnormal hematologic findings, mainly in the lymphocytes, have been reported in an African hedgehog, *Atelerix albiventris*, with gastrointestinal lymphosarcoma [8]. Thromboplastin of the platelets is essential for blood clotting (coagulation). They are non-nucleated disc-like cell fragments of the megakaryocytes in mammals [11,12].In sub-mammalian vertebrates, the thrombocytes are oval and nucleated [13].

2. MATERIALS AND METHODS

Animals

Twenty adult (12 females and 8 males) long-eared hedgehogs *Hemiechinus auritus* (Fig. 1) were collected from several localities of the city of Benghazi. A pregnant female was obtained from the wilderness and brought to the laboratory. It gave birth to four pups, hedgehoglets (Fig. 2). The adult hedgehogs were examined for abnormalities. Physical examination revealed an absence of apparent disease symptoms such as discharges and absence of damages in the structures.

Figure 1. An adult *Hemiechinus auritus*

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Figure 2. Four one-day-old hedgehoglets.

Chemicals

Component of formalin- acetic acid-alcohol (F.A.A.) fixative solution, Zenker fixative solution and Harris hematoxylin and eosin stains were of technical grade. Anesthetic ether, sodium chloride of the normal physiological saline solution, ethyl alcohol, methyl alcohol and xylene were also of technical grade.

Blood sampling

After anesthetizing the hedgehogs with anesthetic ether, blood samples were obtained from the heart by means of a 19 G needle. The samples were kept in plastic tubes containing the anticoagulant heparin**.**

Preparation of stained blood smears

The collected heparinized blood was used to prepare blood smears according to the steps that were outlined by [14]. The dried blood smears were fixed by dipping them into absolute methanol for three minutes. The glass slides were put into a glass carrier and then dipped into a glass container of Harris' haematoxylin for five minutes. The next step was to wash with distilled water for one minute followed by dipping into a container of tap water for ten minutes. The slide was then kept for two minutes in jars containing eosin stain. Distilled water was employed to wash the stained smears for one minute. The slides were dried at room temperature. Canada balsam was the mounting medium for the glass coverslips

Microphotography

The stained sections were closely examined under the light microscope to investigate the developing micromorphological aspects of the blood cellular elements. Selected fields of the stained blood films were microphotographed with the aid of a light microscope- digital camera unit. A personal computer was connected to the digital computer. With the aid of a T-V capture built-in card and a Movie Maker software program, the selected photographs were captured and saved in a portable storage device. The PhotoManager program was employed to adjust color, brightness and contrast of the captured microphotographs. The magnification power of the adjusted figures was determined by using a micrometer stage slide to measure the distance between lines as appeared with the used objective lens.

3. RESULTS

Leucocytes

These granular leucocytes were the most predominant cells in the stained blood films of both the newborn and adult long-eared hedgehogs. Their cytoplasm, whose neutrophilic fine granules were not easily visible, looked light pink in color. The nucleus of both the newborn (Figs. 3, 4) and adult (Figs. 5,6,7,8) hedgehog's neutrophils showed polymorph lobulation. Hematoxylin-eosin stained blood smears of the newborn animals revealed the presence of four to seven nuclear lobes.

Neutrophils

On the other hand, neutrophils of the adult animals had nuclei with four to eight lobes. The separation of the lobes from each other and the joining threads were in most cases very obvious. However, in some nuclei, the lobes looked like a band and the nuclear threads were not clear, as in Figure 3 of the newborn and Figures 5 and 7 of the adult animals.

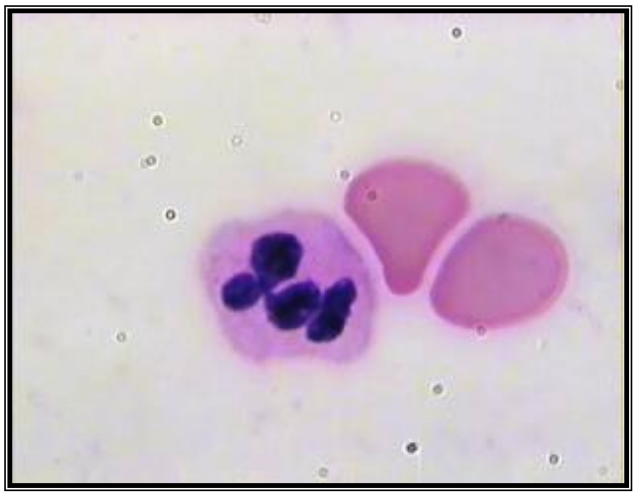

Figure 3. Hematoxylin-Eosin stained blood smear of a newborn *Hemiechinus auritus* showing a four-lobed nucleated neutrophil. (X 1500)

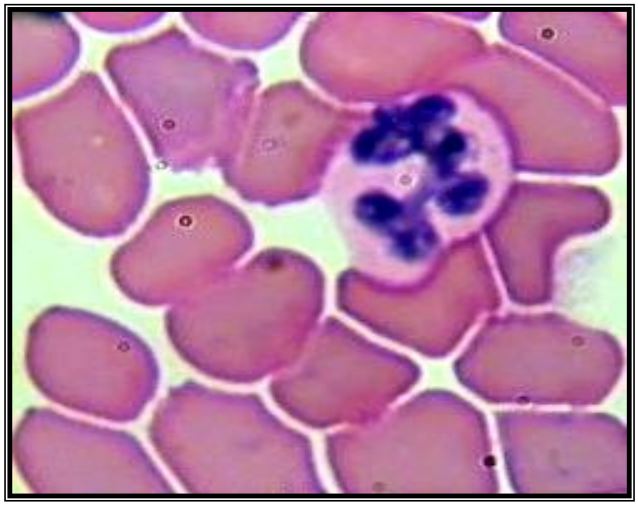

Figure 4. A neutrophil with seven nuclear lobes in a blood smear of a newborn long-eared hedgehog. (H-E, X 1500)

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Figure 5. Blood smear of an adult animal stained with hematoxylin-eosin. A neutrophil whose nucleus has four lobes. (X 1500)

Figure 6. A neutrophil with eight nuclear lobes of different sizes in a blood smear of an adult animal. (H-E, X 1500)

Figure 7. A multi-lobed nucleated neutrophil of an adult long-eared hedgehog. Some of the lobes are fused forming bands. (H-E, X 1500)

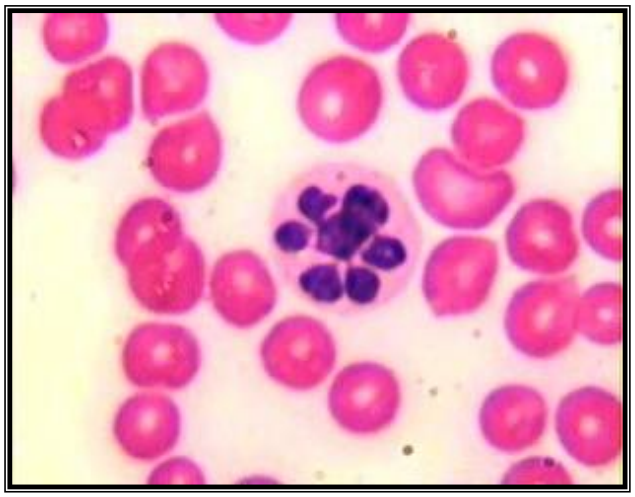

Figure 8. Stained blood smear of an adult animal showing a neutrophil with a peculiar distribution of its nuclear lobes. Two big lobes are closely related and the other five small lobes are attached to them. (H-E, X 1500)

Eosinophils

Eosinophilic granules were easily observed in the cytoplasm of the eosinophils of the newborn and adult hedgehogs. The nucleus of the eosinophils had the appearance of horseshoe (Fig. 9), bilobed (Fig. 10) or trilobed (Fig. 11) in blood films of the hedgehoglets. The eosinophilic leucocytes of the adult hedgehogs showed bilobed (Fig. 12) and trilobed (Fig. 13) nuclei. In Figure 13, there was a very small lobe connected to the joining thread of two lobes.

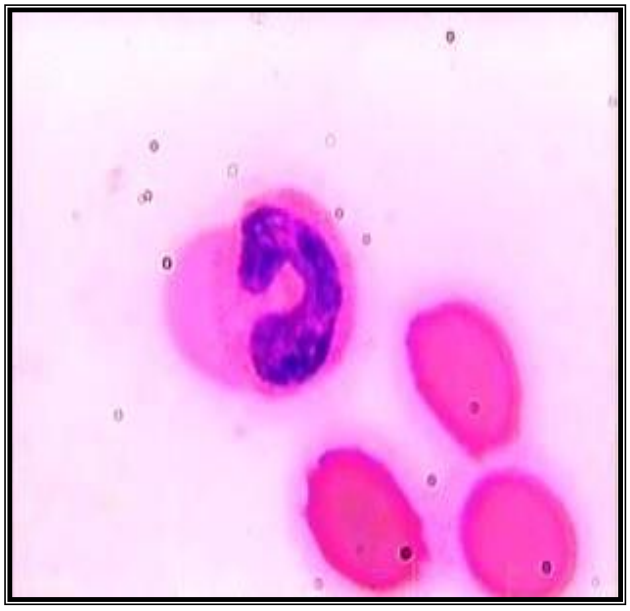

Figure 9. Blood smear of a newborn hedgehog showing an eosinophil whose nucleus appears as a horseshoe. (H-E, X 1500)

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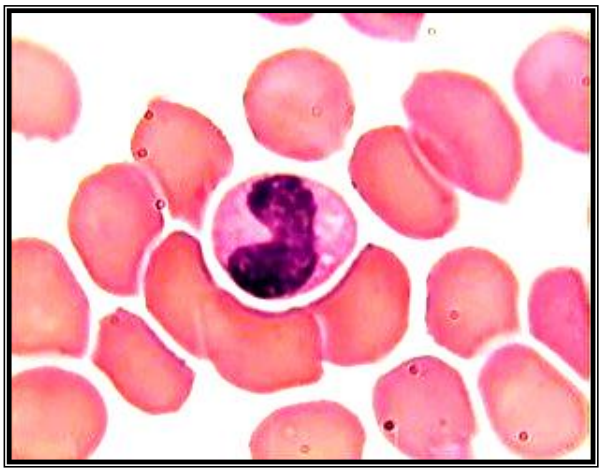

Figure 10. An eosinophil with bilobed nucleus in a blood film of a newborn animal. (H-E, X 1500)

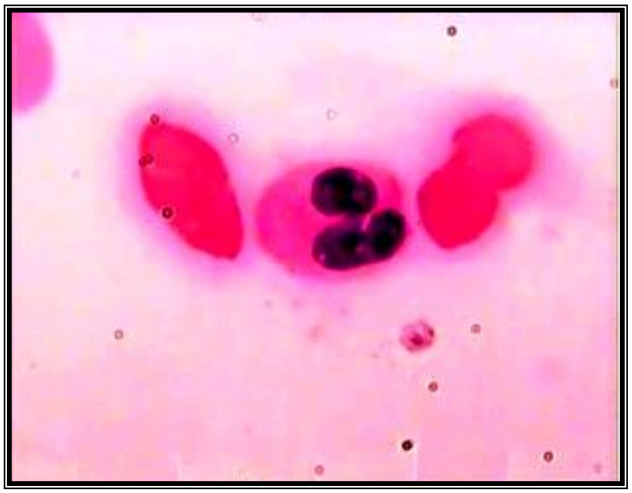

Figure 11. Hematoxylin-eosin stained blood smear of a newborn long–eared hedgehog. A three-lobed nucleated eosinophil is observed. (H-E, X 1500).

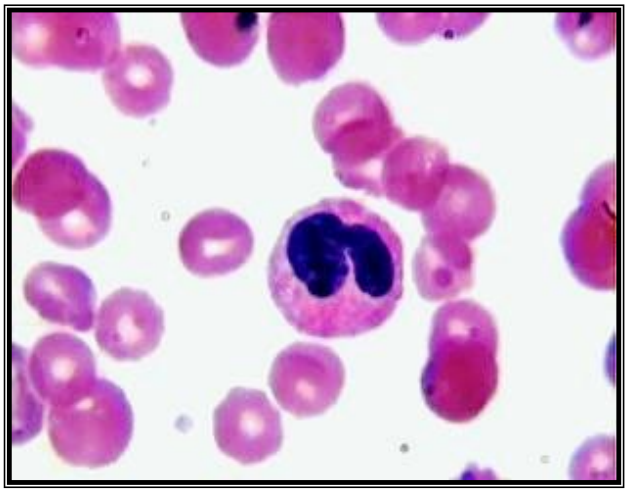

Figure 12. An eosinophil with bilobed nucleus in a stained blood film of an adult hedgehog. (H-E, X 1500)

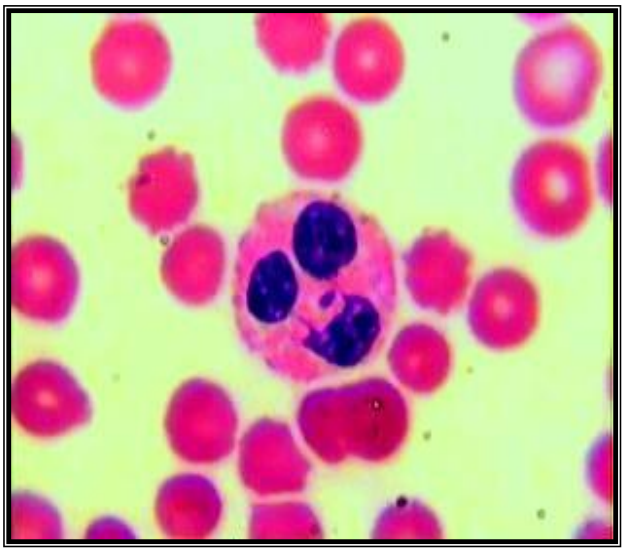

Figure 13. Stained blood smear of an adult long-eared hedgehog showing an eosinophil whose nucleus is trilobed. (H-E, X 1500)

Basophils

Basophils of the newborn animals had large nuclei filling most of the cell. The coarse large basophilic granules of the cytoplasm obscured the outlines of the nuclei resulting in the irregular appearance of the nuclear borders (Fig. 14). Figure 15 shows a basophilic leucocyte of an adult hedgehog. The nucleus was large. The basophilic granules interfered with the clear appearance of the nucleus.

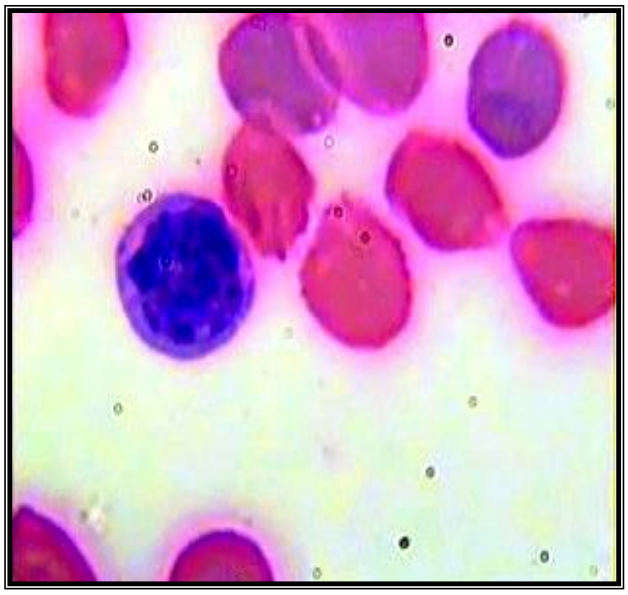

Figure 14. Basophilic granular leucocyte of a newborn long-eared hedgehog. The nucleus is large with irregular borders. (H-E, X 1500)

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Figure 15. A Basophil in a blood film of an adult hedgehog. The nucleus is large and fills almost the whole cytoplasm. (H-E, X 1500)

Lymphocytes

The majority of the hedgehoglets lymphocytes were small in size and their regular spherical nuclei filled nearly the entire cell volume leaving a narrow rim of cytoplasm (Fig. 16). Small, medium and large lymphocytes were also observed in blood smears of the adult animals. Spherical nuclei of the medium and large-sized lymphocytes leave more cytoplasm space (Fig. 17).

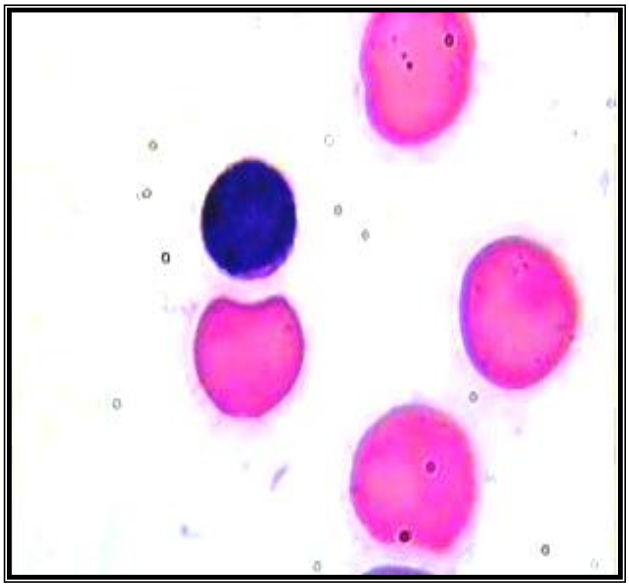

Figure 16. A lymphocyte of a newborn hedgehog. The smooth nucleus filled most of the cell leaving a narrow area of cytoplasm. This figure also shows typical erythrocytes with a flattened discoid appearance. (H-E, X 1500)

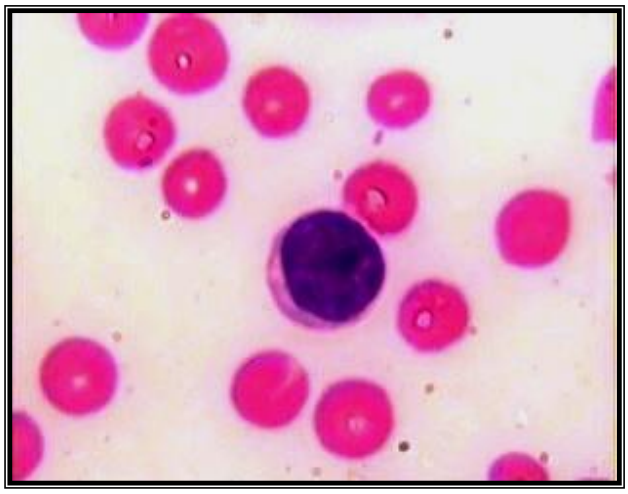

Figure 17. Stained blood film of an adult long-eared hedgehog showing a lymphocyte. The large nucleus leaves a narrow space of cytoplasm. (H-E, X 1500)

Monocytes

The kidney-shaped nucleus of the agranular monocyte of the newborn hedgehog could take an eccentric location leaving a considerable amount of cytoplasm (Fig. 18). In other instances, the nucleus was round, small and eccentrically located. The monocyte of the adult hedgehog had an eccentrically located large nucleus with a deep depression at one side giving the nucleus more or less a horseshoe appearance (Figure 19). Monocytes with large irregular nuclei were also encountered (Fig. 20).

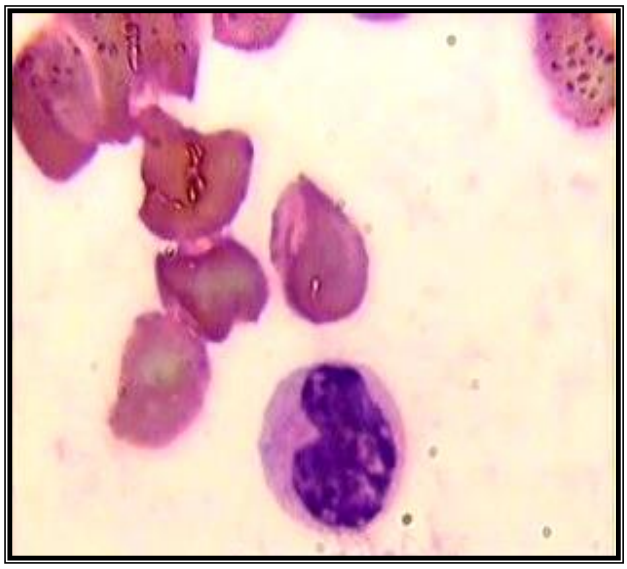

Figure 18. Monocyte in a blood smear of a newborn hedgehog. The nucleus is kidney-shaped. (H-E, X 1500)

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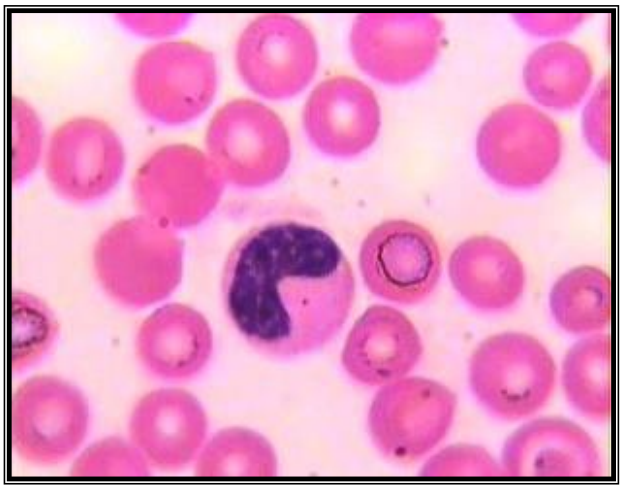

Figure 19. A blood film of an adult hedgehog. A monocyte whose nucleus is eccentrically located shows a deep depression. The discoid non-nucleated erythrocytes are observed with their centrally located thin areas. (H-E, X 1500)

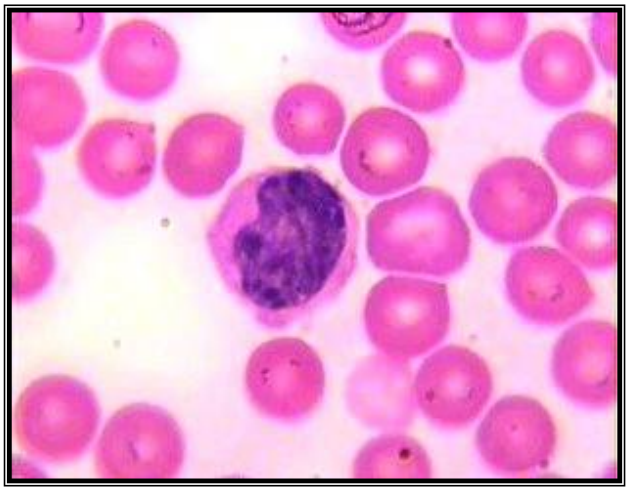

Figure 20. A monocyte in adult hedgehog blood film. The nucleus exhibits an irregular shape. (H-E, X 1500)

Platelets

Figures 21 and 22 showed platelets in blood films of newborn animals. The platelets appear round to oval in shape. A centrally located dark area surrounded by a less dense area could be observed. They showed linear arrangement or grouped into aggregates. The non-nucleated cytoplasmic fragments of the megakaryocytes in the blood smears of the adult hedgehog had a morphological appearance similar to that of the newborn platelets (Figs. 23,24).

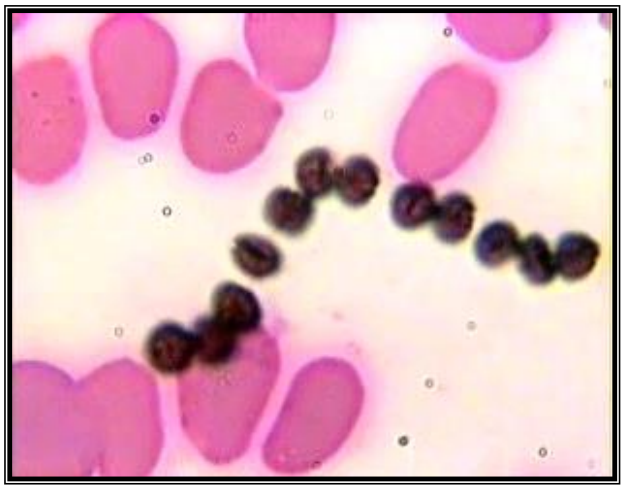

Figure 21. Platelets in a blood smear of a newborn hedgehog. They are oval to rounded in shape. They show a linear arrangement. (H-E, X 1500)

Figure 22. An aggregate of the oval to rounded in shape platelets of a newborn long-eared hedgehog. Centrally located dark areas are observed. (H-E, X 1500)

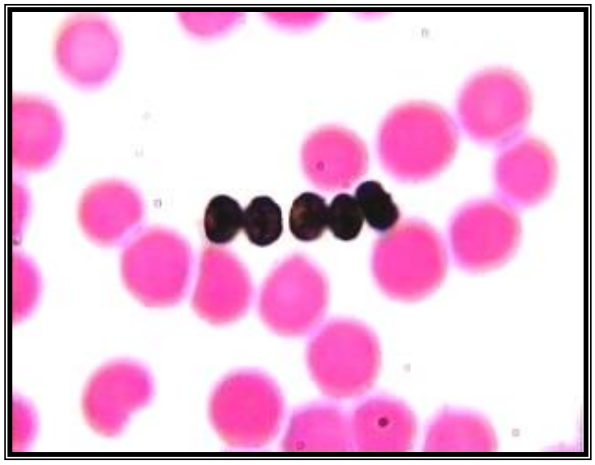

Figure 23. Oval to rounded platelets of an adult long-eared hedgehog. The linear arrangement has a small number of platelets. (H-E, X 1500)

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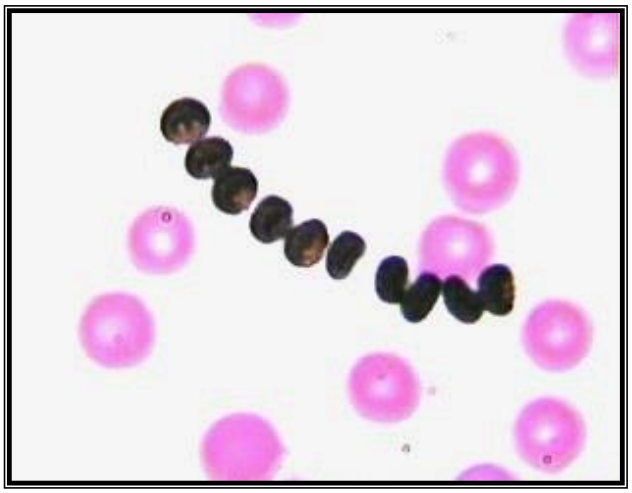

Figure 24. Platelets of an adult hedgehog showing a linear arrangement. They show centrally located dark areas. (H-E, X 1500)

4. DISCUSSION

As in other mammalian species, stained blood smears of the long-eared hedgehog revealed that the cellular elements include, granulocytes, agranulocytes, non-nucleated platelets and non-nucleated discoid erythrocytes. Neutrophil nuclear lobulation of the newborn *Hemiechinus auritus* reached the maximum of seven lobes, whereas nuclear lobulation of up to eight lobes was observed in neutrophils of the adult animals (Fig. 6). Joining threads of the nuclear lobes and cytoplasmic features did not show any sign of peculiarity in neutrophilic cells of both newborn and adult hedgehogs[15]. The drumstick-like appendage (Barr body) representing the inactive X chromosome that has been observed in neutrophils of human females [16] could not be observed in blood smears of the female newborn and adult hedgehog.

Eosinophils with non-lobulated nuclei were reported [16]. Results of the present investigation confirmed the presence of such type of eosinophils in the blood of the adult long-eared hedgehogs. Results also showed that nuclear trilobulation was frequent in eosinophils of the newborn and adult hedgehogs. Such trilobulation was described as rare in eosinophils of human beings and was not referred to in other mammals [17]. The peculiar appearance of the basophils was limited to the large irregular nucleus in newborn hedgehogs. Basophils with a large nucleus was observed in adult animals. Outline irregularity as well as bilobulation were noticed in basophils of other mammals [17].

In both the newborn and adult animals the lymphocytic agranulocytes had almost identical morphological features. Most of the lymphocytes were small in size. Medium and large-sized cells were also observed. In equine, swine and canine species small lymphocytes constitute the majority of the lymphocyte population. A similar trend has been observed in human blood [18].

The variable shapes of the eccentrically located nuclei of the monocytes were observed in the blood smears of the newborn and adult long-eared hedgehogs. Monocytes with kidney-shaped, horseshoe, oval and round nuclei have been noticed in the blood of some other mammalian species [19].

The non-nucleated round to oval platelets of the newborn and adult hedgehogs had regular outlines. The observed centrally dark area surrounded by less dense area was reported in human platelets and in platelets of other non-human mammals. The biconcave nature of the non-nucleated discoid erythrocytes of the newborn and adult long-eared hedgehogs was identical to that of some other mammals including man [20]. On the other hand, blood erythrocytes of pigs and ruminant mammals have a flattened discoid appearance. Species of the *Camelidae* family have non-nucleated ovoid erythrocytes [21].

5. CONCLUSIONS

National records on several aspects of wildlife animals are vital. Such records may give adequate knowledge of the biological parameters that help in understanding the features of the well-being of these organisms in their natural environment. This study provides identification of the morphological characteristics of different peripheral blood cells of the long-eared hedgehog species as a reference for future hematological studies of this species and may be used as a basis for comparison in clinical cases.

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