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Comparative anatomical and histological study of spleen in cat (*Felis catus*) and caucasian squirrel (*Sciurus anomalus*)

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Abstract

This work aimed to identify the morphological and histological structures of the spleen in the Cat (F. catus) and the Caucasian squirrel (S. anomalus). Eight adult animals of both sexes were used in this study. The weight of the animals was estimated to be between 2.57-4.37 kg in Cat *F. catus* and 190.3-225.7 g in Caucasian squirrel *S. anomalus*. The spleen in *F. catus* and *S. anomalus* appears dark reddish brown; the shape of *F. catus* is elongated, tongue-like, with one end widened and tri-rayed. While in the Caucasian squirrel *S. anomalus*, it appears as tongue-shaped and rounded at both ends, concave on one side, and smaller in size than the spleen of the Cat *F. catus*. The spleen was found in the abdominal cavity on the left side of the stomach in both studied animals. This work revealed that the spleen in Cat *F. catus* and the Caucasian squirrel S. anomalus is encased in a thick capsule of connective tissue containing collagen fibers, smooth muscle fibers, blood vessels, nerves, and Fibroblasts. In addition, an outer layer of mesothelial cells covers the capsule. This work also showed that the splenic tissue in the cat (*Felis catus*) and the Caucasian squirrel (*Sciurus anomalus*) has white and red pulps. The former is composed of splenic nodules, the peripheral lymphatic sheath (PALS), and the marginal zone. Furthermore, the splenic nodules are distributed randomly throughout the splenic tissue, with many shapes (spherical, irregular, and oval), and appear singly or in clusters of 2-3 nodules. The results also detected the presence of central arterioles in splenic nodules.

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Introduction

The domestic cat (*F. catus*) belongs to the family Felidae and the order Carnivora, a type of mammal that humans have had for 4000 years. Domestic cats have a strong, flexible body, sharp teeth, and claws adapted to catch small prey. They are covered with fine, soft fur (wool) in various colors (1). The Caucasian squirrel (*Sciurus anomalus*) (mountain rat) belongs to the Sciuridae family and the Rodentia order. There are about 50 genera and 273 identified species of squirrels. It is a mammal that lives in trees and has wide eyes and round ears; it is also characterized by a long tail with thick, fluffy hair that reaches approximately 50-80% of the body length. The fur is soft, reddish-brown or chestnut-brown, and gray-brown on the back. Furthermore, the fur on the belly and lower parts is yellowish-gray (2). The spleen is the largest of the lymphatic organs, located between the stomach and the left kidney and diaphragm (3-5). It is a thin, spongy lymphatic organ with a dark red to bluish-black color (6). Furthermore, it is the largest blood filter in the body due to the presence of active phagocytic cells, through which it filters the blood from old, weak, and dead cells (7). The spleen, called the graveyard of red blood cells, analyzes and stores iron in the form of hemosiderin in its phagocytic cells, then transports it to the bone marrow. In addition, it is worth noting that red blood cells die before they reach the spleen, not in it, but are buried in it (8). Interestingly, the spleen is essential in the immune system because it contains lymphatic nodules that produce lymphatic white blood cells, and it includes clumps of white blood cells known as lymphocytes

that release special proteins into the blood. These proteins-antibodies-fight bacteria, viruses, and any other substances that cause infection (9,10). Moreover, the spleen, along with the liver, contributes to red blood cell production during embryonic development and loses this function after birth. It is also considered a primary blood reservoir, storing blood in a concentrated form and releasing it into the bloodstream during emergencies, such as bleeding. Moreover, it controls blood flow in the blood vessels (6). In general, the spleen has two surfaces: one convex and the other concave, through which blood, lymphatic vessels, and nerves enter from the middle side via the hilus into the splenic tissue. The capsule encases the spleen in dense fibrous connective tissue containing elastic fibers, collagen fibers, and smooth muscle fibers (8,10). Histologically, two areas can be distinguished in the spleen: the white and red pulps, which differ in function and shape. The white pulp is a group of lymph nodules called splenic nodules, and each nodule contains a germinal center, a lymphatic sheath surrounding the central artery, and a marginal zone (11,12). In addition, the white pulp has a regular histological structure. It is considered a natural reservoir for the accumulation of migrating B and T lymphocytes, with some free macrophages present. These cells are aggregated around a small artery called the central arteriole, which is one of the branches of the trabecular arteries that enter the splenic parenchyma and are surrounded by lymphatic tissue to form the white pulp (13,14).

The second area of the spleen is the red pulp, which surrounds the splenic nodules; it consists of a network of fine reticular fibers that contain spaces filled with blood. It consists of two distinct structures: splenic sinuses and splenic cords. Splenic sinuses, also called venous sinuses, are specialized parts of the blood vessels that connect the splenic arteries to the veins. Whereas the splenic cords, also called cords of Billroth, are cords of loose connective tissue that do not contain endothelial cells, unlike venous sinuses, which include distinct, clear endothelial cells (15,16).

This work provides basic information on the morphology and histology of the spleen in F. catus and S. anomalus, given the lack of studies in the Iraqi environment.

Materials and methods

Ethical approval

Ethical approval was obtained and accepted from the scientific research ethics committee of the College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq, numbered EC-74 on 12 /1/ 2025.

Samples collection

This study used eight adult animals of both sexes: four of Cat *F. catus* and four of Caucasian Squirrel *S. anomalus*. The weight of the animals was estimated to be between 2.57- 4.37 kg in Cat *F. catus* and 190.3-225.7 g in Caucasian Squirrel *S. anomalus*. Samples from the local market in Baghdad governorate were purchased, and the animals were classified using the field directory of wild animals in Iraq (17). Regional anesthesia was induced by Chloroform and dissected by longitudinal incisions, removing the spleen.

Histological preparations

The samples were fixed in 10% formalin for 24 hours. The specimens were processed in an ascending series of alcohols (70%, 80%, 90%, 100%) and cleared in xylene before embedding in paraffin wax blocks. A microtome was used to cut paraffin wax blocks into sections 5-6 µm thick; the sections were stained with Harris Hematoxylin and Eosin. Then, we examined the slides under a microscope equipped with a digital camera (Canon, Japan) to photograph the selected spleen sections (18).

Results

The morphological description of the spleen

The results of the morphological characteristics of the current study indicated that the spleen in Cat *F. catus* and Caucasian squirrel *S. anomalus* appears dark reddish brown in color, the shape of Cat *F. catus* is elongated shape resembling a tongue, with one end widened and tri-rayed, While in Caucasian squirrel *S. anomalus* it appears as a tongue-shaped and rounded at both ends, concave on one side, and smaller in size than the spleen of Cat *F. catus* In addition, the spleen is located in the abdominal cavity on the left side of the stomach in the two studied animals (Figure 1).

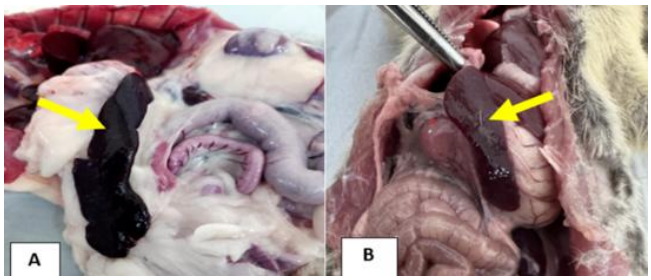

Figure 1: Photomicrograph of the spleen in A. Cat (*F. catus*) and B. Caucasian squirrel (*S. anomalus*).

The histological description of the spleen

According to this work, the spleen in Cat *F. catus* and Caucasian Squirrel *S. anomalusis* is encased by a thick capsule of connective tissue consisting of collagen fibers, smooth muscle fibers, blood vessels, nerves, and Fibroblasts. In addition, the capsule is surrounded by an outer layer of peritoneal mesothelial cells (Figures 2 and 3). Many septa extend from the capsule into the splenic tissue; they are composed of smooth muscle fibers, collagen fibers, blood vessels, nerves, and Fibroblasts (Figures 2 and 3). The results of the current study observed that the spleen in Cat *F. catus*

and Caucasian Squirrel *S. anomalus* consists of two distinct regions, the white pulp and the red pulp. The white pulp consists of the splenic nodules, peripheral lymphatic sheath (PALS), and marginal zone (Figures 4-7).

Additionally, the splenic nodules are randomly distributed throughout the splenic tissue and range in shape from spherical to oval to irregular. The splenic nodules in Cat *F. catus* are larger and more widespread than in the Caucasian Squirrel *S. anomalus*, and appear singly and sometimes in clusters, consisting of 2-3 nodules (Figures 4-6). The nodules may also appear clustered in the Caucasian Squirrel *S. anomalus* spleen, but with a lower proportion. The Splenic nodules consist of the germinal center, a group of cells including macrophages, lymphocytes, and plasma cells, and the presence of the germinal center is critical for increasing the immune response. Moreover, this study identified central arterioles in splenic nodules, although they are not centrally located and may be peripheral.

In contrast, the splenic nodules in the *S. anomalus* contain 1-2 central arterioles (Figure 8), while in the *F. catus* they contain 1-3 central arterioles (Figure 7). In addition, the central arteriole is surrounded by a fibrous sheath (PALS). The present study noted the presence of a clear, distinct marginal zone in the splenic nodules, which is the last edge of the white pulp, separated from the red pulp. The marginal zone is seen in the *F. catus* (Figure 7). At the same time, it cannot be distinguished more clearly in the *S. anomalus* (Figure 8). The second distinct area in the spleen is the red pulp, a vascular reticular lymphoid tissue composed of numerous blood vessels, venous sinuses, and splenic cords with reticular fibers, lymphocytes, and macrophages (Figures 9 and 10).

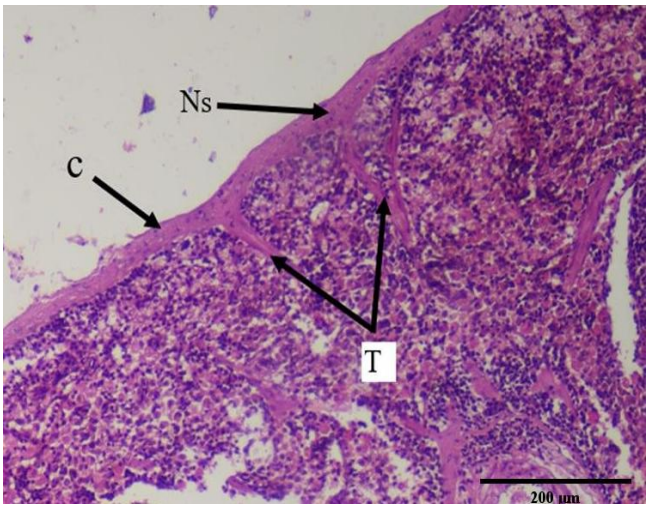

Figure 2: Photomicrograph of the spleen in a cat (*F. catus*) showing: (C) Capsule, (Ns) Nucleus of smooth fibers, (T) Trapeculae (H&E, 4X).

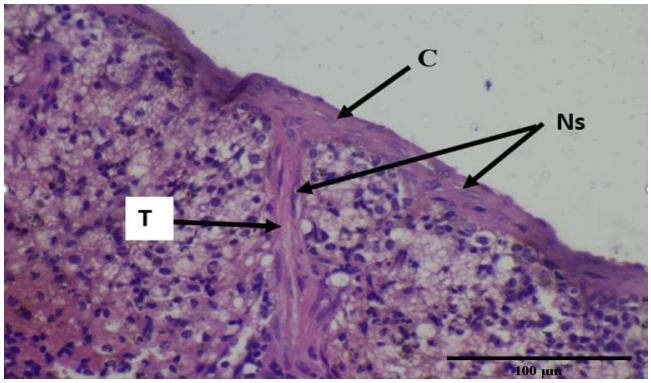

Figure 3: Photomicrograph of the spleen in *S. anomalus* showing (C) Capsule, (Ns) Nucleus of smooth fibers, (T) Trapeculae (H&E, 10X).

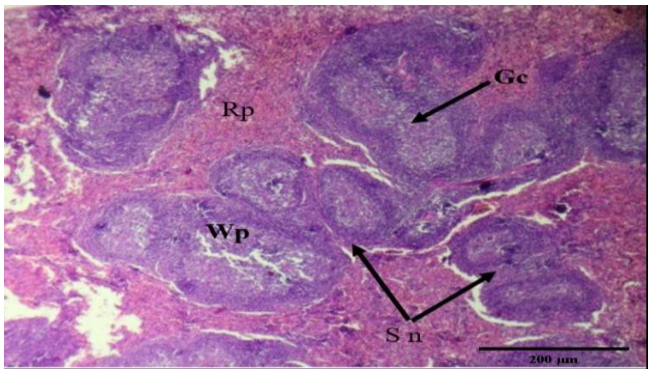

Figure 4: Photomicrograph of the spleen in a cat (*F. catus*) showing: (Wp) White pulp, (Rp) Red pulp, (Gc) Germinal center, (Sn) Splenic nodule. H&E, 4X).

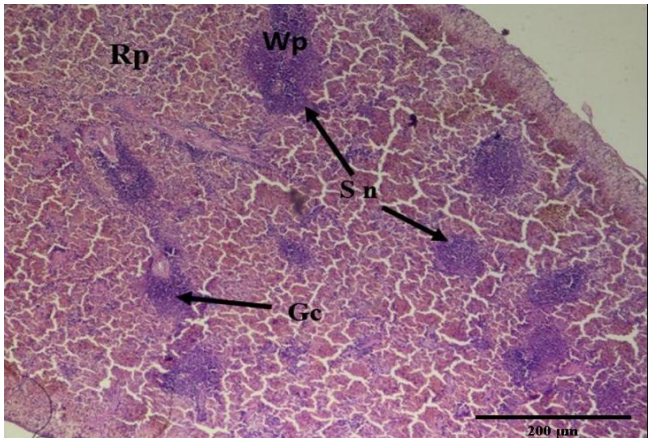

Figure 5: Photomicrograph of the spleen in *S. anomalus* showing (Wp) White pulp, (Rp) Red pulp, (Gc) Germinal center, (Sn) Splenic nodule (H&E, 4X).

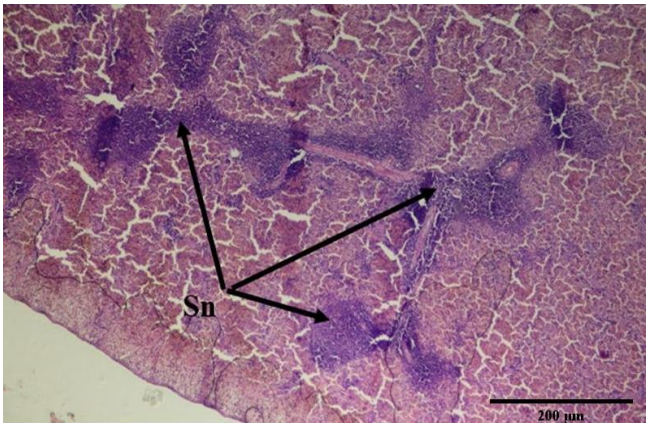

Figure 6: Photomicrograph of the spleen in *S. anomalus* showing (Sn) Splenic nodule (H&E, 4X).

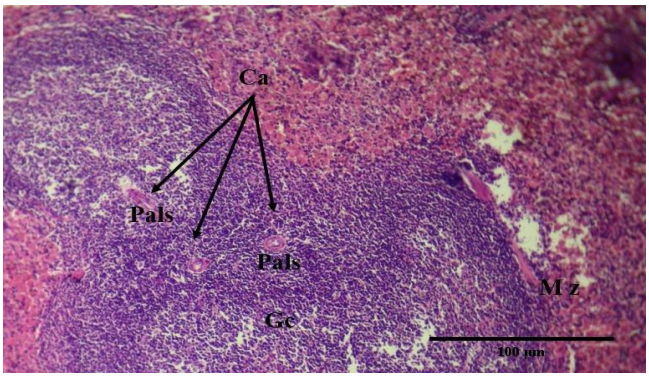

Figure 7: Photomicrograph of the spleen in a cat (*F. catus*) showing: (Gc) Germinal center, (Ca) Central arterioles, (Pals) Periarteriolar lymphatic sheath, (Mz) Marginal zone (H&E, 10X).

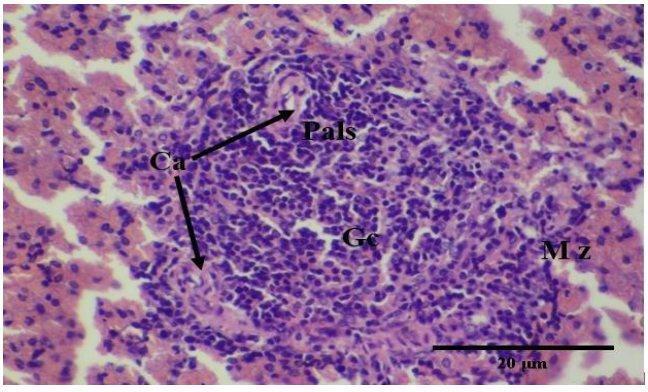

Figure 8: Photomicrograph of the spleen in *S. anomalus* showing (Gc) Germinal center, (Ca) Central arterioles, (Pals) Periarteriolar lymphatic sheath, (Mz) Marginal zone (H&E, 40X).

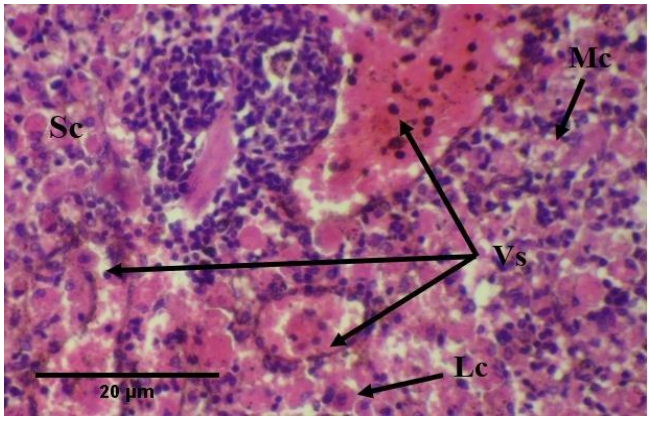

Figure 9: Photomicrograph of the spleen in a cat (*F. catus*) showing: (Mc) Macrophages, (Lc) Lymphocytes, (Sc) Splenic cord, (Vs) venous sinuses (H&E, 40X).

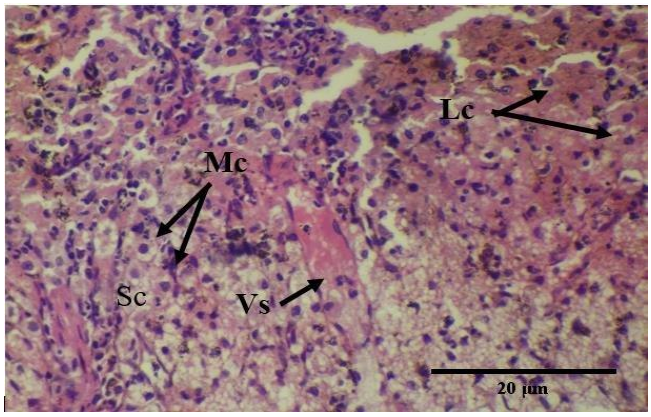

Figure 10: Photomicrograph of the spleen in *S. anomalus* showing (Mc) Macrophages, (Lc) Lymphocytes, (Sc) Splenic cord, (Vs) venous sinuses (H&E, 40X).

Discussion

The findings of the Morphological description revealed that the spleen in the Cat *F. catus* and the Caucasian squirrel *S. anomalus* appears dark reddish brown in color. The shape of *F. catus* is elongated, resembling a tongue, with one end widened and tri-rayed. In S. anomalus, it appears tongue-shaped and rounded at both ends, concave on one side, and smaller than the spleen of *F. catus*. Interestingly, a previous study (18) on Weasel (*H. javanicus*) found that the spleen was reddish-brown, elongated, and compressed on both dorsal and ventral surfaces. In contrast, in the Eastern gray squirrel (*S. carolinensis*) it appeared elongated and tongue-like.

In contrast, in the fox (*Vulpes bengalensis*), the spleen is dark brown and tongue-like (19), while in the Hedgehog (*Hemiechinus auritus*) the spleen is bluish-red and tongue-like (20). In the giant African rat (*Cricetomys gambianus*),

the spleen appears as a long, flat slipper from the dorsal-ventral side (21). In contrast, it seems to be a small, elongated, tongue-like shape in the rabbit (*Oryctolagus cuniculus*). At the same time, in guinea pig (Caviaporcellus) is quadrilateral in shape, dark red to blackish in color (22); while the spleen of mouse appears as anarrow ribbon - like (23), spleen of the golden hamster was an elongated lanciform reddish-brownstructure composed of a body and two ends arched cranially and straight caudally and two surfaces: visceral and parietal (24). In fact, the shape and size of the spleen differ among animals, and its red color is due to the accumulation of blood vessels in its tissues.

The results of the current study showed that the spleen in Cat *F. catus* and Caucasian squirrel *S. anomalusis* are surrounded by a thick capsule of connective tissue consisting of collagen fibers, smooth muscle fibers, blood vessels, nerves and fibroblasts, the capsule is surrounded by an outer layer of mesothelial cells of the peritoneum, these results are in agreement with the capsules of Weasel (*H. javanicus*) and Eastern gray squirrel (*S. carolinensis*) (18), White Yorkshire Pig (*Sus scrofa*) (25), rodent (9). On the other hand, the capsule in foxes (Vulpes bengalensis) is composed of dense connective tissue containing collagen, elastic fibers, and a few smooth muscle fibers (19). Additionally, the study indicated that the capsule in the adult albino rabbit spleen is composed of dense connective tissue, and no smooth muscle fiber was observed (13), and this is in agreement with (20) observation that found in Hedgehog (*Hemiechinus auritus*) and giant African rat (*Cricetomys gambianus*) (21), rat (26). The capsule of African Palm Squirrel (*Epixerus ebii*) is composed of thin layers of regular connective tissue that are composed entirely of collagen fibres and fibrocytes (27). Trabeculae extend through the spleen tissue and are similar to capsule tissue because it is an extension of the inner layer of the capsule. This result confirms the literature Weasel (*H. javanicus*) and Eastern gray squirrel (*S. carolinensis*) (18), Hedgehog (*Hemiechinus auritus*) (20), White Yorkshire Pig (*Sus scrofa*) (25), African Palm Squirrel (*Epixerus ebii*) (27). At the same time in rats (28), which indicated the presence of a small quantity of smooth muscle fibers in the septa and smooth muscle fibers in the capsule and septa, proving the spleen's ability to expand and store a large amount of blood, as well as its ability to contract rapidly.

The current study observed that the spleen in Cat *F. catus* and Caucasian Squirrel *S. anomalus* consists of two distinct regions, the white pulp and the red pulp. The white pulp consists of splenic nodules, the peripheral lymphatic sheath (PALS), and the marginal zone. The splenic nodules are randomly distributed throughout the splenic tissue and range in shape from spherical to irregular. The splenic nodules in Cat *F. catus* are larger and more widespread than in the Caucasian Squirrel *S. anomalus*, and appear singly and sometimes in clusters, consisting of 2-3 nodules. The nodules may also appear clustered in the *S. anomalus* spleen, but in a lower proportion. And, these results are consistent with study of Weasel (*H. javanicus*) and Eastern gray squirrel (*S. carolinensis*) (18), Fox (Vulpes bengalensis) (19), rabbits (29), Mice (30,31), Hedgehog (*Hemiechinus auritus*) (20), the observation of study in goats proves the findings of our work, as the splenic nodules appear individually and sometimes in clusters of 2-3 nodules (32).

The Splenic nodules consist of the germinal center, a group of cells including macrophages, lymphocytes, and plasma cells, this result is consistent with the results of previous studies Weasel (*H. javanicus*) and Eastern gray squirrel (*S. carolinensis*) (18), Rabbit (Oryctologus Cuniculus) (22) mice (33), the splenic nodules in the *S. anomalus* contain (1-2) of central arterioles, and this result is consistent with Weasel (*H. javanicus*) and Eastern gray squirrel (*S. carolinensis*) (18), African Palm Squirrel (*Epixerus ebii*) (27) studies, while in the *F. catus* they contain 1-3 of central arterioles. The central arteriole is surrounded by a fibrous sheath (PALS). This study agrees with previous studies on Hedgehog (*Hemiechinus auritus*) (20) and Rabbit (Oryctolagus cuniculus) (22,34), as the marginal zone is clearly detected in *F. catus*. In contrast, the study of African Palm Squirrel (*Epixerus ebii*) (27) is consistent with the *S. anomalus* result in the lack of clarity and distinction of the marginal zone, which lacks separating the white from the red pulp, enabling the germinal center to approach the blood circulation in the red pulp, thus raising the effectiveness of the antigens. This work also indicates the efficiency of the immune system in *F. catus* due to the distinction and abundance of white pulp in the spleen tissue more than in *S. anomalus*, which plays an essential role in removing old and damaged red blood cells (35); this study is in agreement with Fox (*Vulpes bengalensis*) (19), Hedgehog (*Hemiechinus auritus*) (20), and African Palm Squirrel (*Epixerus ebii*) (27) studies. The red pulp in the *F. catus* and *S. anomalus* is classified as a sinusoidal spleen due to the large number of venous sinuses distributed within the splenic tissue, and this is consistent with the study of Hedgehog (*Hemiechinus auritus*) (20). In contrast, the spleen is classified as non-sinusal in mice (36,37).

Conclusions

This study concluded that the spleen was found in the abdominal cavity on the left side of the stomach in both studied animals. The spleen in *F. catus* and *S. anomalus* is encased in a thick capsule of connective tissue. This study also showed that the splenic tissue in *F. catus* and *S. anomalus* has white and red pulps. The former is composed of splenic nodules, the peripheral lymphatic sheath (PALS), and the marginal zone. The results also detected the presence of central arterioles in splenic nodules, where their number in the cat is 1-3 and in the squirrel is 1-2. The Ethical approval number is: EC-74 on 12 /1/ 2025.

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Conflict of interest

The authors declare no conflicts of interest.

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دراسة تشريحية ونسجية مقارنة للطحال في القط والسنجاب القوقازي

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الخلاصة

هدفت الدراسة الحالية الى التعرف على التركيب المظهري والنسجي للطحال في القط .والسنجاب القوقازي. استعملت في الدراسة الحالية ثماتية حيوانات من كلا الجنسين. تتراوح أوزان الحيوانات بين ٢,٥٧ - ٤,٣٧ كغم في القط و١٩٠,٣- ٢٢٥,٧ غم في السنجاب القوقازي. يظهر الطحال في القط والسنجاب القوقازي بلون بني محمر غامق، وبشكل متطاول يشبه اللسان متوسع النهاية من احد أطرافه ويكون بشكل ثلاثي الشعاع في القط، اما في السنجاب القوقازي يظهر بشكل اللسان ومستدير من النهايتين ويكون مقعر من احدى الجهتين واصغر حجما من طحال القط، يقع الطحال في التجويف البطني في الجانب الأيسر من المعدة في الحيوانين موضوع الدراسة، أوضحت نتائج الدراسة الحالية أن الطحال في القط والسنجاب القوقازي محاط بمحفظة سميكة من نسيج ضام يتألف من الياف مغراوية والياف عضلية ملساء وأوعية دموية وأعصاب واورومات ليفية فضلا عن أن المحفظة تحاط بطبقة خارجية من الخلايا المتوسطة، أظهرت نتائج الدراسة الحالية أن نسيج الطحال في القط والسنجاب القوقازي يتألف من منطقتين متميزتين هما اللب الأبيض واللب الأحمر، يتألف اللب الأبيض من العقيدات الطحالية، الغمد اللمفاوي المحيطي والمنطقة الحافية. تنتشر العقيدات الطحالية في نسيج الطحال بشكل عشوائي وتتخذ أشكالا مختلفة كروية، بيضوية وغير منتظمة الشكل، وتظهر بشكل منفرد وأحيانا تكون بشكل تجمعات من ٢- ٣ عقيدات.