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Anatomical, Histological, Histochemical, Scanning and Transmission Electron Microscopic Studies on Water Buffalo (Bubalus Bubalis) Spleen

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ABSTRACT: Immunity in water buffalos stands unique properties, by which they withstand unpleasant environments. Spleen influence buffalo immunity through its hematopoietic and immunological roles. Because of that, we emphasized the normal anatomical, histological, and ultrastructural properties of the water buffalo spleen. We collected twenty spleens randomly from apparently healthy slaughtered water buffalos, aged 3-5 years, and weighed about 400-500 kilograms. We injected latex to highlight the splenic artery and vein branches. General histological and histochemical stains applied to splenic tissues and examined by light microscopy. On the ultrastructure level, spleen subjected to scanning and transmission electron techniques. Results revealed that the spleen of water buffalo is supported externally by thick fibromuscular connective tissue capsule which vascularizes by subcapsular sinus. Stromal trabeculae emerge from the capsule, entering splenic parenchyma, and we noticed it in two forms: avascular trabeculae and vascular trabeculae. Anatomical, light, and scanning findings proved that the splenic artery penetrates the splenic capsule and descends with vascular trabeculae as a trabecular artery until it ends as penciller artery. The parenchyma of water buffalo spleen differentiates into white pulp, which organizes into the periarterial lymphatic sheath along with lymph nodules, red pulp ropes in splenic sinuses among splenic cords, and marginal zone in which macrophage occupies great importance. The present work aims in-depth to study water buffalo spleen structure as a mechanism that can easily diagnose hematopoietic and immunological conditions and enable other researchers to develop effective vaccines and to regulate various diseases.

KEYWORDS: Water buffalo, spleen, anatomy, histology, ultrastructure, and scanning microscope

I. INTRODUCTION:

The effective role of the spleen as a hematopoietic and immunological organ, especially for meat-producing animals such as ruminants makes many researchers interested in discussing the improvements, differences, and clinical scope of the spleen to hemiparasite, anemic crisis, warehousing, and other relative conditions [1]. Bovine spleen entitled as a crucial organ taking part in the defense against hemiparasitic diseases as babesiosis [2]. Notably, it is assumed that the autoimmune reaction to platelet antigens evolves in the spleen [3] and is the ideal one to ascertain the type of the immune response that motivates this autoimmune disorder [4].

Highlighting animal histology and ultrastructural details not only offers an insight into organ activities but also describes in-depth human characteristics even if lower mammalian tissues are accessible. Many researchers, therefore, lately motivated to document the related histological characteristics [5]. Spleen stands for the major secondary lymphatic organ. Normal spleens comprise white pulp (WP), marginal zone (MZ), and red pulp (RP). The lymphoid tissue of WP is composed of 2 parts: the thin periarteriolar lymphoid sheath (PALS) which is rich in T lymphocyte, contrary to lymphoid follicles (LFs) that are composed mainly of B lymphocyte. On the other hand, the marginal zone includes the area that separates white pulp from the red one. Meanwhile, the red pulp is represented by splenic cords and sinuses, as reported in cattle [6], in humans [7] and yak [8].tructurally, the spleen

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specializes in immunological defense, as it filters, retains, and deals with the Bloodborne pathogens in white pulp [9]. Besides, it acts as a pool of red blood cells and platelets during periods of unusual demand in the red pulp [10] in camel. Meanwhile, in the marginal zone of the spleen, the bloodstream throws across an accessible system of reticular cells and fibers in which a variety of myeloid and lymphoid cells are sited. Featuring by macrophages that are perfectly positioned to identify pathogens besides their ability to sieve the blood through distinctive mixtures of pattern recognition receptors. Other immune cells, like natural killer cells (NKC) and dendritic cell (DC), are similarly testified to perform a starring role in the protective immunity via secreting the inflammatory cytokines [11]. The pattern by which cells organize is a key issue in our guard against blood-borne hazards as humans [6]. The clinical and managing immunotoxicity signals may be linked with apparent splenic morphological alterations. Society of Toxicological Pathology issued a guideline for best practices to evaluate the lymphoid organ separately via using descriptive rather than interpretive terms to diagnose changes within these parts, aiming to improve the sensitivity and specificity of lymphoid organ-related changes. That is why the spleen assessed the potential target for treatment in toxicology and carcinogenicity studies [12].

Aim of work:

This current work aimed to achieve an accurate and effective assessment of the immunological activity on the water buffalo spleen, in addition to the general anatomical, histological, histochemical, and electron microscopic examination, which are essential to interpret any specific splenic disorder. Moreover, it would help other investigators categorize disease, treatment, and vaccination strategies.

II. MATERIALS AND METHODSTissue collection

The splenic tissues utilized for this research are assembled from the Munib Municipal Abattoir, Giza Government. A total of 20 spleens of each sex (10 male and 10 female), elderly 3-5 years, and weighing 400 to 500 kilograms had been selected from apparently healthy slaughtered water buffalos without any symptoms of sickness. We collected the samples randomly and at once transported through ice boxes toward the Veterinary Histology laboratory, University of Cairo, Egypt for gross investigation.

The samples labeled promptly, which has borne a code range of ulterior identity. Each sample bathed in tap water on a dissection receptacle. Blood combined with blood clots detached as some distance as viable. Then we divided samples according to the type of examination into three groups:

1-Gross Anatomical Study (Corrosion casting)

The samples selected, kept for about 48-72 h in a refrigerator and casts of the splenic artery and veins were made by injection with latex at the department of Anatomy, Veterinary Medicine Cairo University. The **specimens left in a mixture of 10% formalin and 1% glycerin for seven days before the routine dissection.**

2-Light microscopic examination:

A- General histological examination:

The protocol for histological preparations is as described by Usende et al. [13]. Briefly, water buffalo splenic samples were sliced to yield 3-4 mm thick samples. Then, they fixed by immersion in 10% neutral buffered formalin (10% NBF). The samples are dehydrated in increasing concentrations of ethanol, cleared in xylene, and embedded in paraffin. The paraffin blocks are sectioned with a microtome at (4-6μm) thickness and positioned on slides treated for Hematoxylin and Eosin stain to examine general tissue structure, Masson's trichrome stain for an exhibition of collagenous fibers coupled with smooth muscle fibers, Wiegert's elastic stain for elastic fibers, Gomori's reticulin method for reticular ones and finally toluidine blue stain for mast cell identification [14].

B- Histochemical examination:

Perl's Prussian blue stain highlighted the sites of hemosiderin and ferric salts in the splenic [15]**.** Microscopic observations performed with a Leica microscope (CH9435 Hee56rbrugg) with different magnifications and photomicrographs taken with a computer-enabled digital camera.

ELECTRON MICROSCOPIC EXAMINATION

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A-Scanning electron microscopic examination:

Tissue samples of the water buffalo spleen fixed at once by double fixation in glutaraldehyde with OsO4 or in freshly depolymerized paraformaldehyde (also buffered by phosphates). The samples dehydrated by alcohol and amyl acetate gradual series. Then the samples were dried by the method of the critical point of CO2 determination and galvanized with gold [16] and examined with a DSM- 950 (Zeiss-option - Oberkochen) scanning electron microscope at Regional Center for the Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

B-Transmission electron microscopic examination:

Small tissue blocks from various parts of spleen tissues from water buffaloes fixed in paraformaldehyde-glutaraldehyde in phosphate buffer. Specimens were post fixed in 1% osmium tetroxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), then dehydrated in gradual ethanol and embedded in open Araldite mixture. Semithin sections (1μm), stained with toluidine blue and examined with a light microscope. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate [17] and examined with a JEOL 1010 transmission electron microscope at Regional Center for the Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

III. RESULTS

The spleen of water buffalo in the current study had an average length of 43.7 to 45.5 cm and a width of 14.5 to 15.5 cm while the thickness at the middle part of 2.6 to 2.9 cm and weighing fresh about 1.515±0.03 kg. It had a bright purple color, elongated elliptical with dorsal broad border, and narrow rounded ventral end while the caudal border was convex and slightly higher than the concave cranial border that contained the hilus in its proximal point.

The splenic artery of water buffalo divided into extra parenchymatic three main branches, dorsal (Fig.1.4), intermediate (Fig.1.3), and ventral (Fig.1.2), for each corresponding area of the spleen. The dorsal branch extended caudo-dorsally in the parenchyma for about 2-3 cm and bifurcated into two branches (Fig.1.5) which subdivided into further branches. The intermediate branch divided as the dorsal one supplying the middle segment while the ventral branch considered as the direct continuation of the splenic artery.

The ventral branch of the splenic artery (Fig.1.2) in water buffalo terminated at the ventral extremity with the presence of arterial anastomosis either between the branches of the same artery or between branches of the primary splenic arteries (Fig.1, yellow arrows).

The ventral splenic branch gave off cranial and caudal branches. The cranial branches (Fig.1.8) ranged from 10-12 in number extending in a cranioventral direction to ramify within the corresponding part of the organ. They increased in length as traced ventrally. The caudal branches (Fig.1.9) ranged from 8-10 in numbers with the first branch participating in the nourishing of the middle segment. They extended mostly parallel to each other obliquely in a caudoventral direction.

The splenic vein (Fig.2.1) in water buffalo entered the hilus of the organ proximal to the artery divided into two main branches, dorsal (Fig.2.3) and ventral (Fig.2.2). The ventral splenic branch collecting from the intermediate segment with an additional branch (Fig.2.4). The primary branches of the splenic vein followed the course of the artery, while their further ramifications did not follow the distribution pattern of the artery.

The structural compartments of water buffalo spleen are described using histological, histochemical, and electron microscopic "TEM & SEM" studies. The spleen of water buffalo is a compact organ composed of supporting stroma and functional factory of parenchyma. It gains its support by thick fibromuscular connective tissue capsule which is vascularized by subcapsular sinus (Fig.4A). The overview of scanning studies on water buffalo spleen visualized capsular thickness and the sinus outlined the area underneath (Fig.4B). This subcapsular sinus characterizes by flat endothelial lining cells. Red blood cells, lymphocytes, and other splenic cells flow inside its lumen (Fig.4C).

Stromal trabeculae emerge from the capsule entering splenic parenchyma. It owns two forms: avascular trabeculae

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and vascular trabeculae. The type which appears without blood vessels represents avascular trabeculae that are highlighted by smooth muscle and parallel organization of fibers which are mainly collagen and elastic in type with few reticular ones (Fig. 4D). The other form of trabeculae is those carrying blood vessels and referring to vascular trabeculae with trabecular sinuses (Fig. 4E). The main splenic artery supplies the water buffalo spleen is spotted with light and scanning microscopic examination penetrating the splenic capsule and descending with the vascular trabeculae as trabecular artery (Fig. 5A & B) till it ends as penciller artery (Fig. 5B)

The trabecular artery exists as a medium-sized one having 3 tunics: tunica intima of a thin endothelial layer, tunica media are composed mainly of circularly arranged smooth muscle fibers and collagen fibers, and finally, tunica adventitia which encompasses fibrous connective tissue and vasa vasorum (Fig. 5C). Scanning electron micrograph reveals endothelial slits of a trabecular artery as a small circular opening that encircles by concentric layers of smooth muscle and myofibroblast cells (Fig. 5D).

The parenchyma of water buffalo spleen differentiates into white pulp, marginal zone, and red pulp. The white pulp organizes into two main components: periarterial lymphatic sheath (PALS) and lymph nodules. The PALS developed like a chain at the point from which the lymphocytes aggregates around the trabecular artery of the vascularized trabeculae (Figs. 5B & E). It is supported by a net of large irregular highly branching follicular dendritic cells as provided by SEM examination (Fig. 5E), in addition to many lymphocytes with few red blood cells in between (Fig. 5F).

Meanwhile, the lymph nodule "L.N." appearance in the histological sections mark as rounded or ovoid masses of condensed lymphocytes aggregation and it displays light center and dense periphery. The central arteriole gets it's homing inside the lymph nodule, and it originates from the PALS and representing a branch of the trabecular artery (Fig.6A). Lymphocytes are gathered by follicular dendritic cell networks due to their highly branched cytoplasmic processes (Fig.6B). On the other hand, the lymph nodule is visualized by scanning examination as a ball-like structure formed by irregular rough knobby lymphocytes that connected by a net of highly branching cytoplasmic processes of large-sized follicular dendritic cells (Fig. 6C & D). TEM studies reveal that follicular dendritic cells held large oval euchromatic nucleus and few cytoplasms with ill-developed organelles (Fig. 6E).

The marginal zone "MZ" in water buffalo spleen is found between the lymph nodule and the red pulp. It is rich with red blood cells and marginal sinuses (Fig. 6B & 7A). This area is supported by the cytoplasmic processes of follicular dendritic cells (Fig. 6B). SEM examination revealed the marginal sinus like a channel demarcating MZ between lymphocyte rich lymph nodule and red pulp with high red blood cell content (Fig. 7B). One of the most important cells that appear in the MZ is macrophage cell which showed a positive reaction with pearl's Prussian blue stain. On the contrary, a negative reaction to this stain existed inside the lymph nodule (Fig. 7C). TEM studies recognize the marginal zone cellular structure in a part of which, lymphocytes either B cell of large euchromatic nucleus or T cell of the small heterochromatic nucleus. In the other part, a macrophage cell and high endothelial venule are situated (Fig. 7D). On the ultrastructure level, the macrophage cell perceives a kidney-shaped nucleus and its cytoplasm rich in lysosomes, pinocytotic vesicles, mitochondria, and rough endoplasmic reticulum (Figs. 7E& F).

The last compartment in water buffalo spleen is the red pulp. It comprises two main structures: the first one in which the splenic cells organize in cord-like to form splenic cords and the other is the place where the blood circulate named splenic sinuses. Water buffalo spleen covers the two types of circulation; closed circulation inside the splenic sinuses and open circulation in between the splenic cords (Fig. 8A). SEM clarified the irregular arrangement of splenic cords that are associated with a network of reticular cells or interdigitating cells. These cords consist of lymphocytes, iron crystal-like of hemosiderin pigment, red blood cells, and other splenic cells. Moreover, in between them, small open channels like vessels standing for splenic sinuses are seen (Figs. 8B &C). The blood retains from these sinuses to the large splenic vein (Fig. 8D). The thin branch of a trabecular artery is found sharing its end in the red pulp as penciller artery (Fig. 8E). TEM examination proved the sinusoidal capillaries lined with flat endothelial cells and its lumen occupied by red blood cells (Fig. 8F).

Light microscopic examination showed high endothelial venules (HEV) in the red pulp of water buffalo spleen (Fig. 9A). SEM examination demonstrated the HEV like channel encircled by lymphocytes, rectangle crystal of iron and red blood cells (Fig. 9B). Meanwhile, TEM showed this venule having B and T lymphocytes and the pericytes are observed demarcating its external surface (Fig. 9C). At the ultrastructure level, the T lymphocyte in the HEV highlight by a large heterochromatic nucleus with a thin rim of cytoplasm and few organelles (Fig. 9D). The iron crystal is packed with electron-dense bodies of hemosiderin pigment by TEM (Fig. 9E).

Other splenic cells that were recognized in the red pulp of water buffalo spleen were mast cell, macrophage, natural killer cells, eosinophil, platelets, myofibroblast, and reticular or interdigitating cells. Mast cell highlighted purple specific granules using a toluidine blue stain (Fig. 10A). Meanwhile, this cell was seen at the ultrastructure level containing a centrally located heterochromatic nucleus and electron-dense cytoplasmic granules (Fig. 10B).

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On the other hand, macrophage cells showed the same structure as in the MZ.

Natural killer cells are showed by transmission electron microscope examination with prominent receptors, indented heterochromatic nucleus, and abundant cytoplasm that contain lysosomes and ribosomes (Fig. 10C). Another cell remark with a bilobed heterochromatic nucleus and electron-dense granules referred to leukocytes cell (Fig. 10D). The ultra-structure of the platelets can be distinguished by large numerous alpha granules, small few gamma granules, open canalicular system, and peripheral microtubules with actin filament-forming marginal bundle (Fig.10E).

In the spleen of water buffalo, the red pulp is reinforced by two types of cells: Myo-fibroblast cell and reticular or interdigitating cell. Myo-fibroblast cell by histological examination stands as a long, thin cell with a flat bulged nucleus and a thin rim of cytoplasm (Fig. 11A). By the TEM examination, it appears as a long cell with a flat heterochromatic nucleus and ill-developed cytoplasm. It is squeezed in between red blood cells (Fig. 11B). On the contrary, large euchromatic nucleus and few ill-developed cytoplasmic organelles label the reticular or interdigitating cell (Fig. 11C).

Figures:

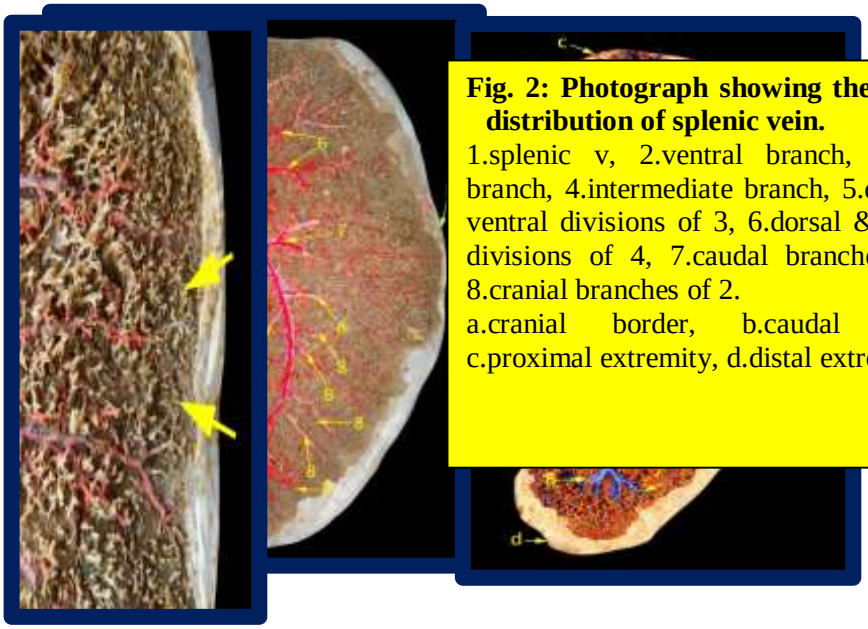

Fig. 2: Photograph showing the venous distribution of splenic vein.
1.splenic v, 2.ventral branch, 3.dorsal branch, 4.intermediate branch, 5.dorsal & ventral divisions of 3, 6.dorsal & ventral divisions of 4, 7.caudal branches of 2, 8.cranial branches of 2.
a.cranial border, b.caudal border, c.proximal extremity, d.distal extremity.

Fig. 3: Photograph showing the terminal arterial anastomosis toward the caudal splenic border (Yellow arrows).

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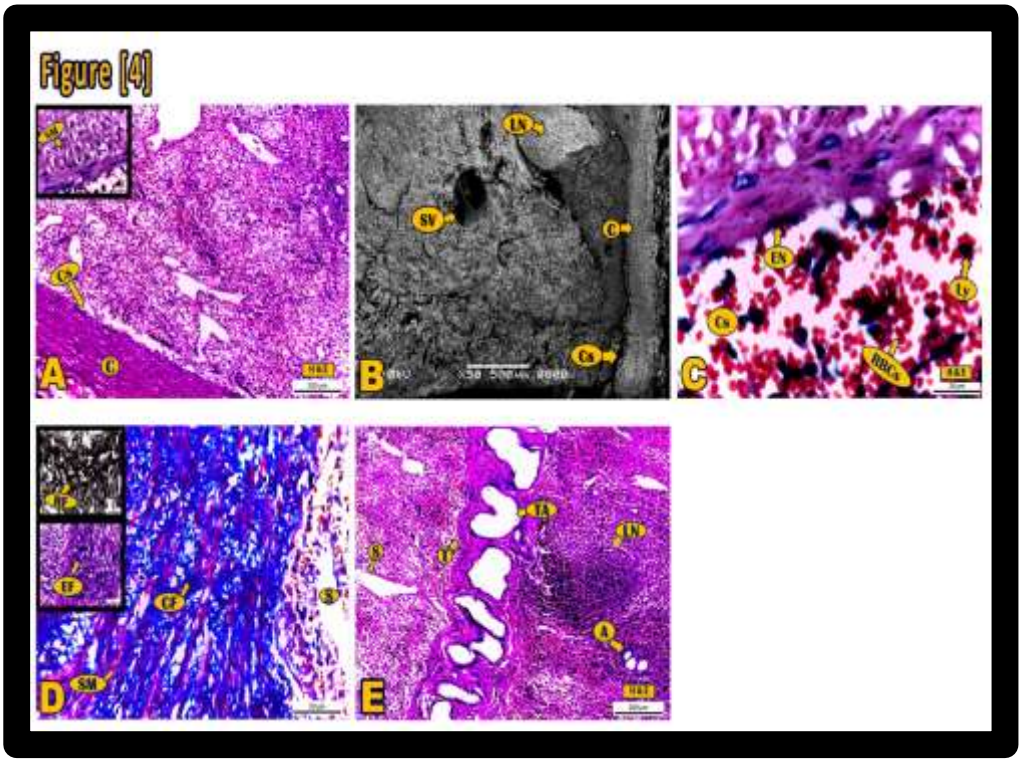

Fig.4: Photomicrograph clarified the stromal elements of water buffalo spleen: **(A) L.M.:** Thick fibromuscular capsule (C) with sub capsular sinus underneath (CS). Notice: the smooth muscle (SM) (inside box H&E X1000) (H&E X100). **(B) SEM**: Thick capsule (C) and sub capsular sinus (CS). Notice the Lymph nodules (LN), splenic vein (SV). (X50). **(C) L.M.:** Subcapsular sinus (Cs) was lined by endothelial cells (EN) and holding red blood cells (RBCs), lymphocytes (Ly) and other splenic cells. (H&E X1000). **(D) L.M.:** Avascular trabeculae consisted of smooth muscle (SM), parallel collagen fibers (CF) (Masson's trichrome stain X400), Elastic fibers (EF) (lower box Wiegert's elastic stain X400) and a few of reticular ones (RF) (upper box Gomori's reticulin method X400).

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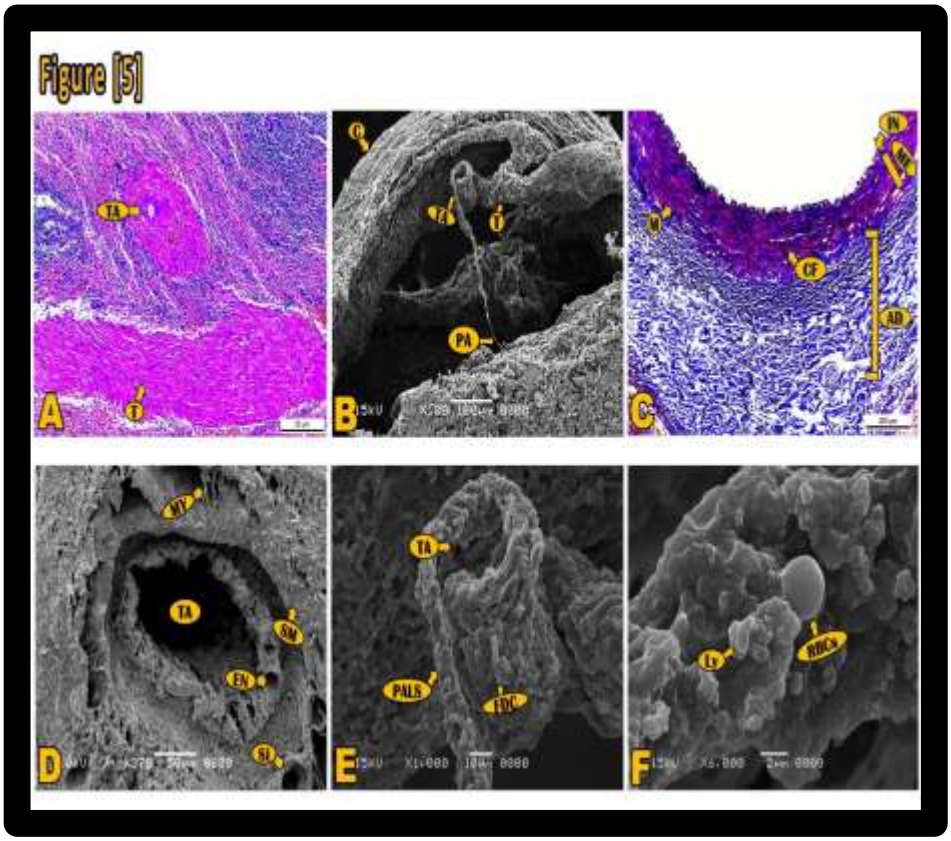

Fig.5: Photomicrograph demonstrated the periarterial lymphatic sheath area of water buffalo spleen: **(A) L.M.:** The beginning of PALS from the trabeculae (T), in which splenic artery pass as trabecular artery (TA). (H&E X400). **(B) SEM:** Splenic artery pathway; from the capsule (C), descend with trabeculae (T) as trabecular artery (TA) and end as penciller artery (PA). (X180). **(C) L.M.:** Structure of trabecular artery as medium sized type consisting of tunica intima (IN), tunica media (ME) of circularly arranged smooth muscle (M) and collagen fibers (CF) and tunica adventitia (AD). (Masson Trichrome Stain X100). **(D) SEM:** Trabecular artery (TA) with its endothelial lining (EN) and surrounded by smooth muscle (SM) and myofibroblast cells (MY). Notice the surrounding circular closed sinus (Si). (X370) **(E) SEM:** PALS originate from

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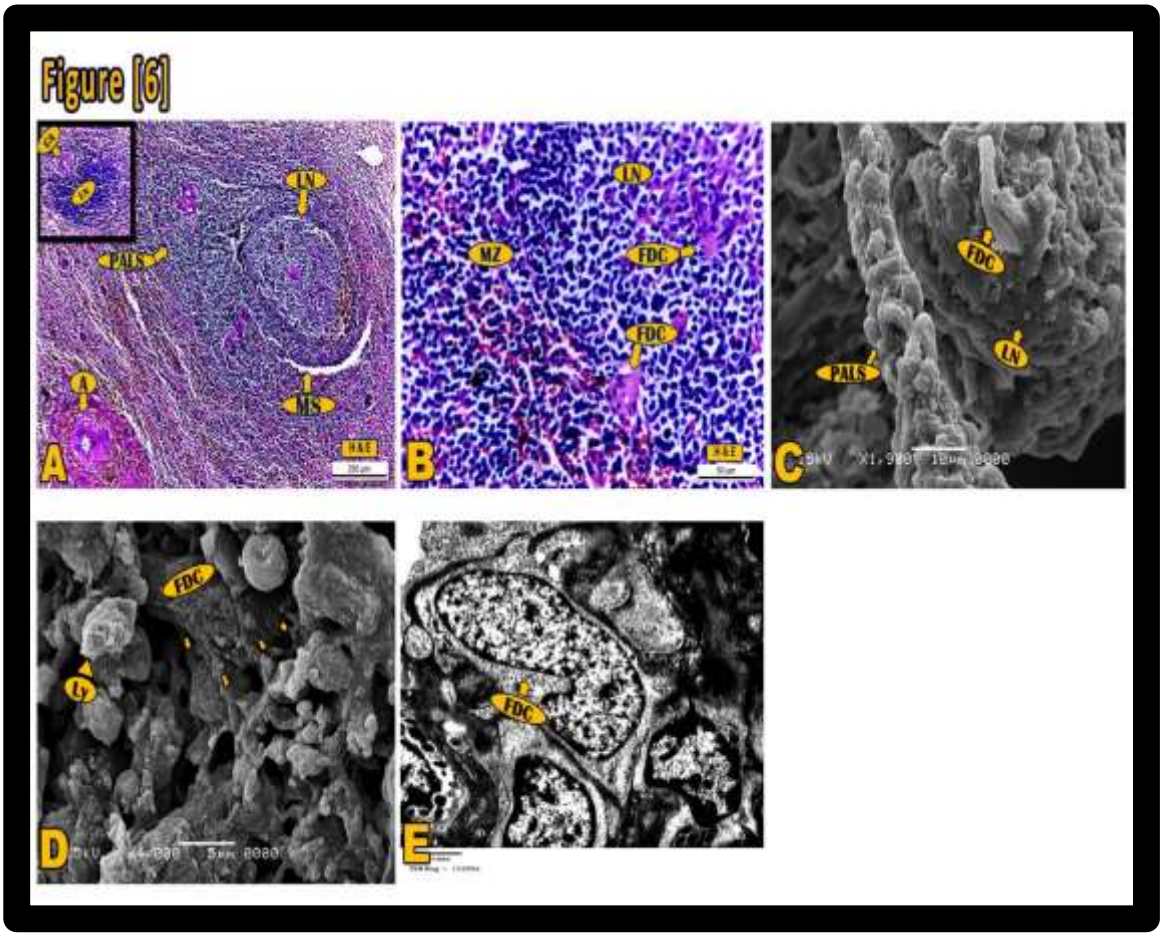

Fig.6: Photomicrograph represented the white pulp area in the spleen of water buffalo: **(A) L.M.:** White pulp area including lymph nodule (LN) contained condensed lymphocytes and the peri arterial lymphatic sheath (PALS). Notice: trabecular arteriole (A) and marginal sinus (MS). (H&E X100). **(B) L.M.:** Follicular dendritic cells (FDC) inside lymph nodule, in addition it appeared in the marginal zone area (MZ). (H&E X400). **(C) SEM:** Ball like lymph nodule (LN) that supported by follicular dendritic cells (FDC). Notice: chain like periarterial lymphatic sheath (PALS). (X1.900). **(D, E)** Photomicrographs illustrating follicular dendritic cell structure. **(D) SEM:** Several cytoplasmic processes (arrows) of large irregular follicular dendritic cells (FDC) and irregular knobby lymphocytes (Ly). (X4.000). **(E) TEM:** Follicular dendritic cells contained large oval nucleus with ill developed cytoplasmic organelles. (Uranyl acetate and lead citrate X10.000).

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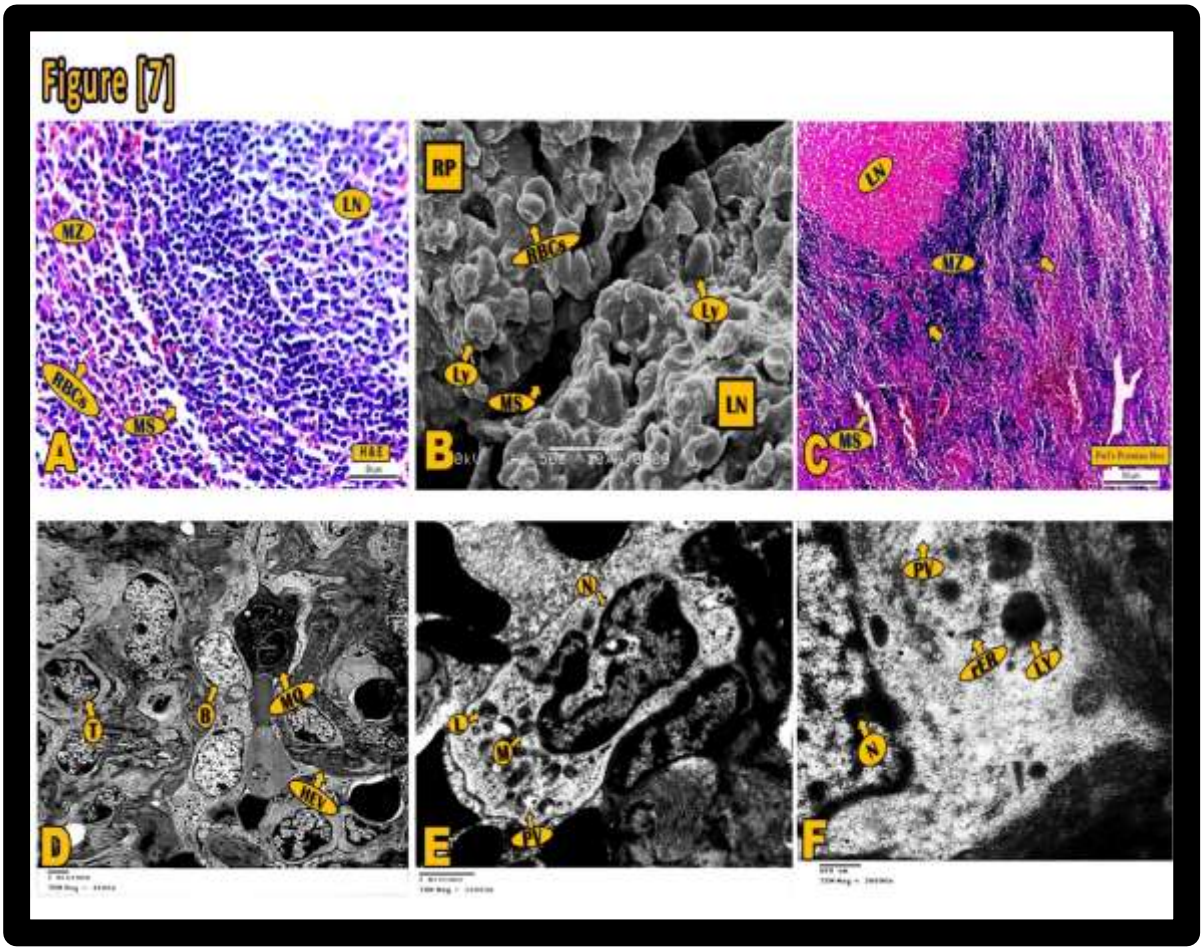

Fig.7: Photomicrograph referred to the marginal zone area of water buffalo spleen: **(A) L.M.:** Lymph nodule (LN) and the marginal zone area (MZ) with marginal sinus (MS) and red blood cells (RBCs). (H&E X400). **(B) SEM:** Marginal sinus (MS) in marginal zone area between the lymph nodule (LN) containing lymphocytes (Ly) and red pulp (RP) with red blood cells (RBCs). (X2.500). **(C) L.M.:** Positive reaction (arrows) inside the macrophage of marginal zone area (MZ) and around the marginal sinus (MS) while negative one inside lymph nodule (LN). (Pearl's Prussian Blue Stain X400). **(D) TEM:** Marginal zone cellular structure in a part lymphocyte either B cell (B) or T cell (T) and in the other part macrophage cell (MQ) and high endothelial venule (HEV). (Uranyl acetate and lead citrate X4.000). **(E, F) TEM:** Macrophage cell with kidney shaped nucleus (N) and cytoplasm was filled with lysosomes (L), pinocytotic vesicle (PV), mitochondria (M) and rough endoplasmic

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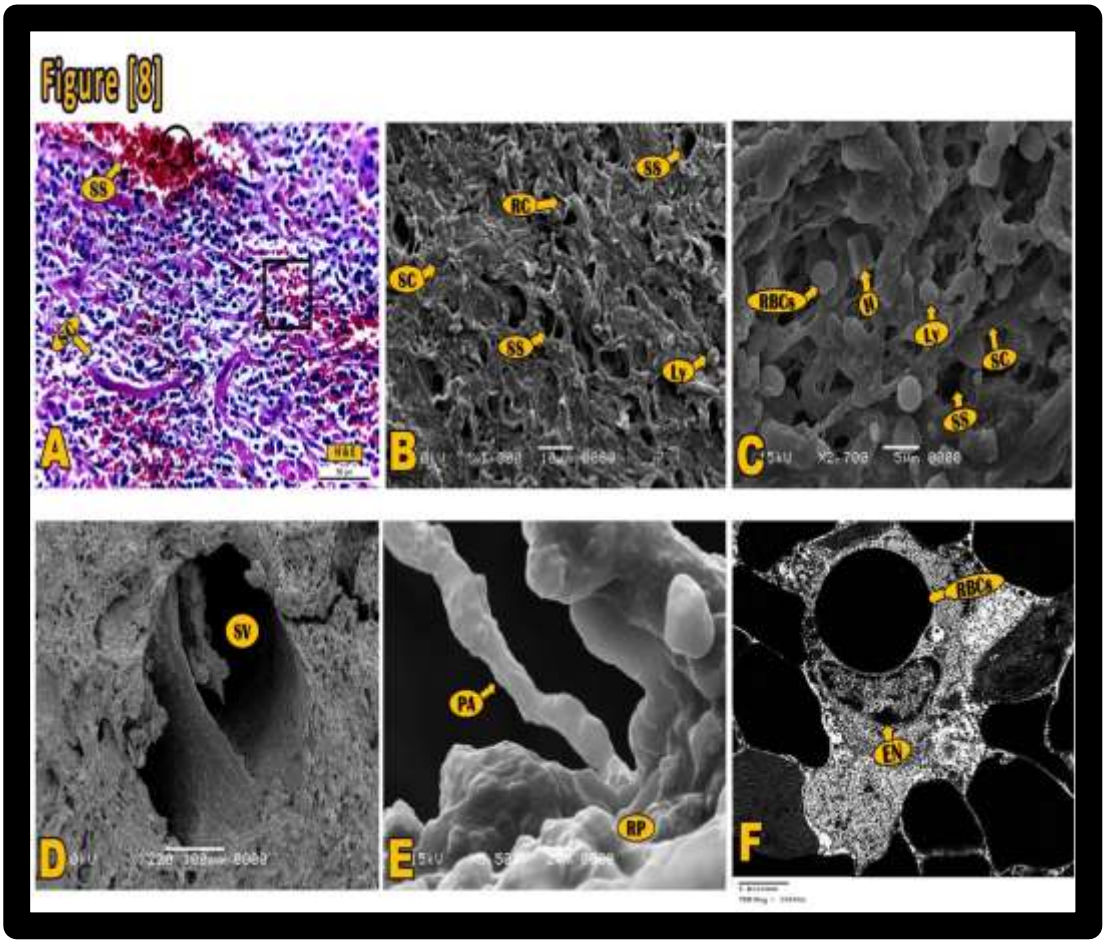

Fig. 8: Photomicrograph revealed the area of red pulp in water buffalo spleen: **(A) L.M.:** Red pulp parts as splenic cords (SC) and splenic sinuses (SS). Notice open circulation (cube) and closed circulation (circle). (H&E X400). **(B, C) SEM:** Splenic cord cells (SC): lymphocytes (Ly), reticular cells (RC), iron crystal of hemosiderin pigment (H) and red blood cells (RBCs) and splenic sinuses (SS). (X 1.300). (X 2.700). **(D) SEM:** Large opening of splenic vein (SV) inside red pulp. (X 220). **(E) SEM:** End part of the splenic artery as penciller artery (PA). (X 5.500). **(F) TEM:** Sinusoidal capillary with its lining endothelium (EN). Notice red blood cells (RBCs) inside its lumen. (Uranyl acetate and lead citrate X 10000).

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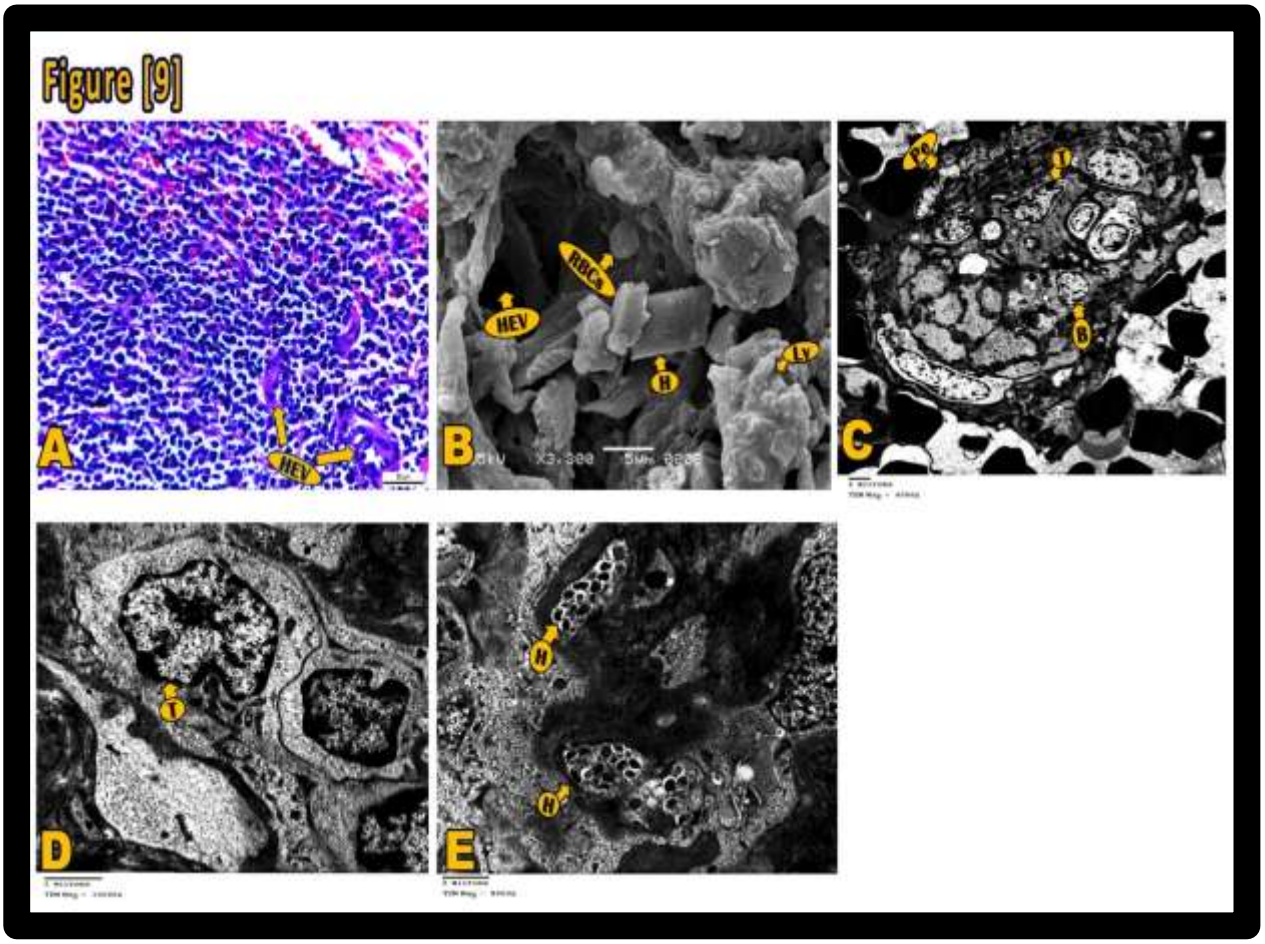

Fig. 9: Photomicrograph highlighting the high endothelial venule (HEV) inside the red pulp of water buffalo spleen: **(A) L.M.:** High endothelial venule (HEV). (H&E X400). **(B) SEM:** HEV encircled by lymphocytes (Ly). Notice iron crystal of hemosiderin pigment (H) and red blood cells (RBCs). (X 3.300). **(C) TEM**: HEV contained B lymphocyte (B) and T lymphocyte (T). Notice pericyte (Pe). (Uranyl acetate and lead citrate X4000). **(D) TEM:** T lymphocyte (T) with heterochromatic nucleus and few cytoplasmic organelles. (Uranyl acetate and lead citrate X10.000). **(E) TEM:** Electron dense bodies of hemosiderin pigment (H) in iron crystal.

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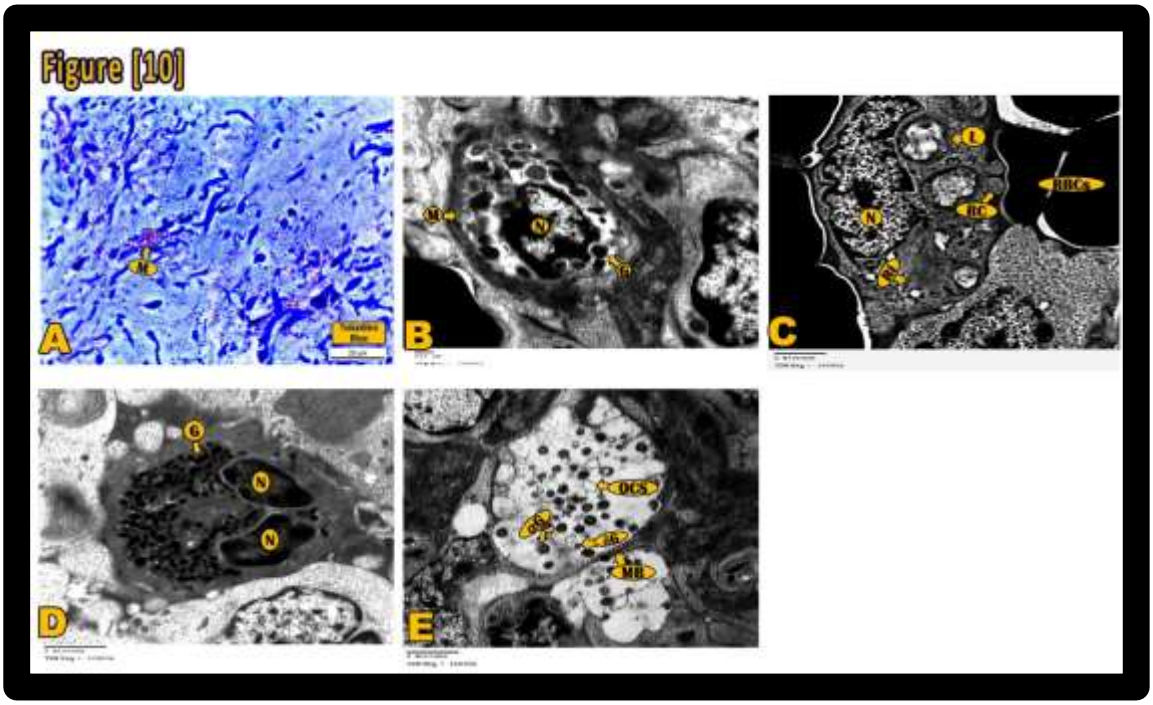
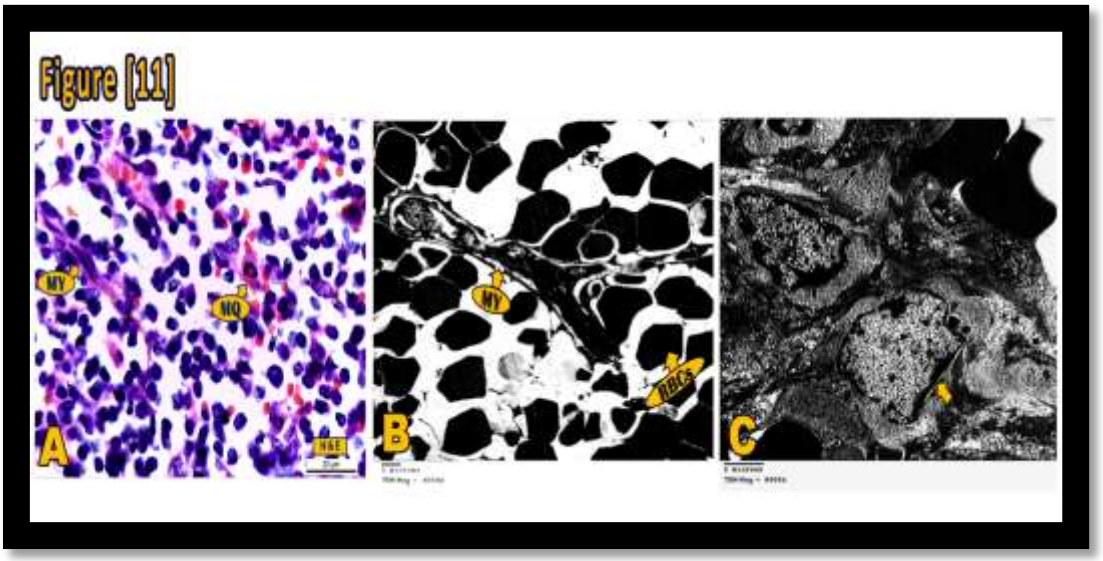


Fig. 11: Photomicrograph representing the supporting cells in the splenic red pulp of water buffalo: **(A) L.M.:** Myofibroblast cell (MY) as long thin cell with flat bulged nucleus and thin rim of cytoplasm. Notice macrophage cell (MQ). (H&E X1000). **(B) TEM:** Myofibroblast cell (MY) appeared as long cell with flat nucleus. Notice red blood cells (RBCs) surrounding it. (Uranyl acetate and lead citrate X 4000). **(C) TEM:** Reticular or interdigitating cell with large euchromatic nucleus (arrow) and ill developed cytoplasmic organelles. (Uranyl acetate and lead citrate X 8000).



IV. DISCUSSION

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The spleen is inquiring persistent concern from anatomical, histological, immunological, and scientific points of view as reported by Fishbeck and Sibastiani [18]. It is a dispensable vital hemopoietic organ [19]. The recognition and integration of each splenic partition stand vital for precise evaluation of the immunological impression on the spleen [20]. Additionally, the functional judgment toward splenic immune reactions can be an imperative approach to appraise immune changes [21].

Our investigation to the anatomical parameters of water buffalo spleen is similar to findings of Noor and Maher [22] in buffalo calf that the spleen has a bright purple color, elongated elliptical with dorsal broad border and narrow rounded ventral end while the caudal border is convex and slightly higher than the concave cranial border that encloses the hilus in its proximal point.

Meanwhile, our results differ from those found by FozFilho et al. [23] in horse and Gupta et al. [24] in sheep where the splenic artery has no extra parenchymal divisions. Nevertheless, simulate the results of Noor and Maher [22] in camel, buffalo calf, and some specimens of sheep that the division of the splenic artery is extra parenchymatic.

The current findings are like that detailed by Ismet [25] in cattle and Noor and Maher [22] in buffalo calf concerning the terminal arterial anastomosis. Although, Osman et al. [26] in buffalo calf reported the division of the splenic artery after gaining the splenic hilus, into two primary branches, dorsal and ventral only. Despite this, Gupta et al. [27] in buffalo stated that in some specimens, the splenic artery bifurcates into two primary branches and in other specimens trifurcate into three primary branches without any anastomosis.

Our observations are in agreement with Noor and Maher [22] in buffalo calf, that along the course of the ventral splenic artery, there were short cranial and long caudal branches, but they do not mention the first emerged branch participating in nourishing of the middle segment of the spleen.

Osman et al. [26] confirmed that many arterial, venous, and arteriovenous anastomoses had been observed in different parts of the organ revealing that the partial splenectomy was not recommended in buffalo. A result that simulated our observations in the terminal arteries but did not meet our observations in veins in this study.

The splenic vein divided into dorsal and ventral branches 6-8 cm before the splenic hilus. A result, which detected by the work in cattle by Ismet [25]. On this basis, they concluded the possibility of partial splenectomy in the buffalo concerning to the venous distribution. While Wilkens and Munster [28] in cattle and Osman et al. [29] in sheep and cattle revealed the division of the splenic vein inside the organ.

The current research revealed that water buffalo spleen is a compact organ that has a combination of stromal supporting system and parenchymal functional factory. Externally, connective tissue capsule covers water buffalo spleen. It distinguishes by a dense and fibromuscular. Similar to results that are recorded in indigenous cattle [30], in goat [1,31], in the cow [32], in camel [19,33], in pig [34], and in buffalo [22]. On the contrary, in pig, the capsule showed thick and thin fibro-muscular nature in different regions [1]. While in sheep, the fibromuscular capsule is thinner [35, 36]. The cause of this difference between animals is back to the degree of contractility performed by this capsule. Which confirms the abundant smooth muscle in our finding aid in more contractile ability [37]. Consequently, buffalo spleen is more suitable for storage purposes [38] as well as its proficiency to withstand severe anemic circumstances [39].

Under the capsule, our investigation posed attention to the subcapsular sinus. It has flat endothelial lining and in the lumen, red blood cells, lymphocyte, and other splenic cells flow inside. By the aid of scanning studies on water buffalo spleen, we visualized the capsular thickness and the longitudinal channel underneath, representing the subcapsular sinus. These are in parallel to the results obtained in camel [33, 40], in sheep and goat [36], and Gazelles [41]. The previous findings illustrate the chief role of the subcapsular sinuses to act like capsular veins in collecting the venous blood from the spleen to the splenic vein. Therefore, it signifies a unique venous return as the blood flows from the venous sinusoids of the red pulp to the peri-trabecular sinuses to the subcapsular sinuses to the splenic vein [40]. We also support the suggestion of Heath and Spalding [42] in the involvement possibility of the subcapsular sinus in erythrophagocytosis and lymphocyte transport.

Thick stromal trabeculae penetrate buffalo splenic capsule and pass into its parenchyma. They pose two forms; first is without blood vessels so being avascular trabeculae. Moreover, inside this form, smooth muscle fibers arise in a parallel organization of collagen and elastic fibers against few perpendicular reticular ones. The other form of trabeculae, those carrying blood vessels referring to vascular trabeculae. These data are in agreement with [1, 34] in pig, [31] in goat, [40] in camel, [43] in mammals, [20] in buffalo and goat, [1,36,44] in sheep and goat, and [42] in Gazelles. The explanation of these variations may donate to species related function [20]. Concerning, fibers homing trabeculae can serve as a rigid framework [1], also the significance of its elastic component may be an explanation for the elevated elasticity of the spleen [1, 40]. We supported the crucial role of smooth muscle in improving the degree of the contraction inside buffalo spleen. In addition to that, spleen symbolizes a blood-

purifying organ, it can gather up to 1/3 the circulating blood volume by its ability to provide the concentration of red cells in the sinuses [45] and can be rapidly emptied [46]. Therefore, smooth muscle concentration may cooperate with a role in the immune defense [47].

The pathway of the splenic artery with light and scanning microscopic examination starts through the splenic capsule and descends with the vascular trabeculae as a trabecular artery until it ends as penciller artery. This observation comes to hand with the records in mammals [48] and Gazelle [41]. We support Brendolan et al. [48] who clarify that the splenic artery exists as large vessel splits into smaller and smaller branches finally ending in the arteriole of the white pulp as well as in the large sinusoids of the red pulp. Curiously, besides their supply role, it may afford a route for lymphocytes going either into or out of the water buffalo spleen compartments [49].

We detect the trabecular artery in some areas as medium-sized one having 3 tunics: tunica intima of a thin endothelial layer, tunica media thru circular arranged smooth muscle fibers, and collagen fibers as well as tunica adventitia in which fibrous connective tissue and vasa vasorum are homing. On the other hand, our scanning images present it as a small circular opening with endothelial slits lining and encircles by concentric layers of smooth muscle and myofibroblast cells. The same findings documented in cats and dogs [50]. We recommended that these arteries may play a role as a filter to regulate the movement of blood cells throughout their existence slits and microfilaments. Moreover, the size of these slits lining may change under various conditions [51].

The parenchyma of water buffalo spleen in our study differentiates into white pulp, marginal zone, and red pulp. In line with previous literature in the mammalian spleen [52,53]. Unlike some authors who considered the marginal zone as a part of white pulp rather than to be a separate compartment [54]. Cesta [55] and Ikpegbu et al. [53] emphasized that the spleen enclose white pulp, which is the primary region for innate and adaptive immune response as well as B-lymphocyte maturation. The previous roles lie on its content near about one-fourth of the body's lymphocytes so easily initiate immune responses. On the other hand, the marginal zone design to check the systemic circulation versus antigens and pathogens and performs a vital position in antigen processing [43, 54, 56-58]. Meanwhile, the red pulp is mostly involved in hematopoiesis, chiefly in neonatal animals, as a packing place for platelets, iron, and, erythrocytes, it also likes blood sieve that eradicates extraneous substance, harmed and effete erythrocytes [52].

By scanning examination, the white pulp in our research organizes into two main components: periarterial lymphatic sheath (PALS) and lymph nodules (LN). At the point from which the lymphocytes aggregate around the trabecular artery of the vascularized trabeculae, the chain-like PALS originate. Also, many lymphocytes with a few red blood cells in between. This is coincidental with the reports in mammals [18, 54, 55, 57] and camel [40]. On the contrary, Van Rees et al. [59] divided the PALS into the inner PALS and the outer PALS. While Stefanski et al. [60] clarified the complexity to identify and appoint a distinction between them using light microscopy. Our results authenticated the importance of PALS to be a spot of lymphocyte traffic in-which the growth of plasma cells and blood antibodies arises [33].

Brendolan et al. [48] added that PALS bounded by the sluggish blood flow of the sinusoids. This observation is identical to the results we report, in which circular closed sinus exists around the PALS in water buffalo spleen. Given that, the presence of these sinuses may account to fit the securing capacity of macrophage cells in this area.

In the current literature, we get more attention on the reticular framework of the PALS area which seems like a network of large irregular highly branching follicular dendritic cells. It explains their critical role in lymphocyte homing and compartmentalization as mentioned by Abed-Muslih and Mirhish [41] in Gazelle. Moreover, T cells intermingle with follicular dendritic cells and passing B cells, which precedes to isotype switching and somatic hypermutation [61]. They also cooperate with the macrophages presented within the sheath and confer on them the ability to prepare antigen for an immune response [62]. Consequently, the alterations which occur in PALS can determine with predictable cellular variances convoyed with an immunomodulatory composite [63].

We investigate the structure of the lymph nodule as a part of the white pulp in the spleen of the water buffalo. It appears in histological sections as rounded or ovoid masses of condensed lymphocytes aggregation. Each mass looks light in its center and dense periphery. In parallel to the researches of Usende et al. [1] in pig, Maina et al. [19] and Zidan et al. [40] in camel, Noor, and Maher [22] in buffalo calf and Gnanadevi [44] in sheep and goat. Devi et al. [31]; Suri et al. [36] and Abed-Muslih and Mirhish [41] in Gazelles. Unlike camel splenic lymph nodule which is either large and spherical [19] or irregular [64] in its shape. Ward et al. [65] cleared that in the central region of the lymph nodule, larger lymphocytes present while in the outer area, the corona or mantle zone encompasses small to medium lymphocytes. After antigenic stimulation, they exploit germinal centers marked by apoptotic cells and tangible body macrophages [55]. On the other hand, this work visualized the lymph nodule of water buffalo spleen by scanning examination as a ball-like structure formed by irregular rough knobby lymphocytes and connected by a net of highly branching cytoplasmic processes of large-sized follicular dendritic cells. These substantiate what mentioned in the horse by Tablin and Weiss [62]. Therefore, the large area of white

pulp has a significant contribution to the explosion activity of lymphocytes and plasma cells, in addition to the production of antibodies [66].

In buffalo lymph nodule, we notice a central arteriole homing inside it. It originates from PALS and represents a branch of a trabecular artery. This finding is identical to data of sheep and goat [31, 33]. Since the follicles stay continual with the PALS at split locations; central arterioles were situated Ward et al. [65]. Rebelatto [12] added that central arteriole divides inside the lymph nodule to follicular capillaries, which create a web in the germinal center and end at the innermost edge of the marginal zone. We recommend their great sharing in the feeding of white pulp capillary beds [67].

This work has further strengthened the confidence that lymphocytes gather by follicular dendritic cell networks creating a framework of splenic lymph nodule in water buffalo. Besides, with the help of the TEM examination of follicular dendritic cells, it characterizes by the large oval euchromatic nucleus and few cytoplasms with ill-developed organelles. This comes in agreement with the finding of Zidan et al. [40] in a one-humped camel. Authors of this paper simulate the distribution of follicular dendritic cells in lymph nodules to their role as antigen-presenting cells through which they present antigen, cluster their respective lymphocytes, facilitate the selection process and clonal expansion [62]. Moreover, they interconnect the marginal sinus with the white pulp, PALS, and the outer sheath of the central artery [68]. Subsequently, we suppose that FDC grants a way for lymphocytes transporting either into or out of the white pulp [49].

The marginal zone in our finding allocates between the lymph nodule and the red pulp. It is rich with red blood cells and marginal sinuses. These findings are in harmony with mammals [55, 68 -70], camel [19], sheep and goat [31], and Gazelle [41]. Also, marginal zones are prominent in goats but not clearly defined in pig [1]. Scanning EM examination in our study reveals marginal sinus of water buffalo spleens like a channel demarcating marginal zone between lymphocyte rich lymph nodule and red pulp with a high red blood cell content. These results are contrary to what detailed in the marginal zone of camel, it has no marginal sinuses [33, 40]. Marginal sinus stands with vessels that nourish capillary beds of PALS along as well as follicles [54]. The sluggishness of the blood flow along with the tight passages among endothelial cells of the sinuses increases macrophages efficiency in terms of recognition and destroying function [48]. The absence of these sinuses could diminish the responsibility of the marginal zone infiltration process, which can justify why blood parasites are the major health crisis in the camel [40].

Transmission EM studies entail the cellular structure of marginal zone in water buffalo spleen as, in a part of which, lymphocyte either a B cell of a large euchromatic nucleus or T cell of a small heterochromatic nucleus and in the other part a macrophage cell and high endothelial venule were situated. These observations are in total agreement with Lokmica et al. [49] who added that the marginal zone is B cell-dependent, also B lymphocytes are not arranged in a follicular form. Because MZ is an essential passage area for cells that are exiting the bloodstream and inserting the white pulp, B cell intends to monitor the systemic circulation for antigens and pathogens. Besides, its role in antigen processing enabling it to be the primary area of innate and adaptive immune responses [43, 54].

We sustain the fact that different cells of water buffalo spleen situate in marginal zone areas having unique properties that affect directly its integrity and function. One of these cells is dendritic one which appears within a network by its cytoplasmic processes. This finding is coincidental with the records in other mammals [71- 74] and pig [34]. Schneder et al. [2] mentioned that dendritic cells organize as a discontinuous honeycomb-like network spanning the marginal zone with a slight regional discrepancy in bovine animals. We can refer that to their essential role in innate immunity as well as antigen detection and presentation to activate the adaptive immune response [43]. Also, we support other findings that discuss their effective integrity in differentiating and surviving B cells into antibody-producing cells [75,76].

The current investigation posed attention to the MZ macrophage cell, which showed a positive reaction with Pearl's Prussian blue stain representing its ferric ions and hemosiderin pigment content. On the contrary, a negative reaction to this stain seen inside the lymph nodule. These results were consistent with Zidan et al. [40] in camel, Cesta [55] in mammals, and Usende et al. [1] in goat and pig. They trap affected red blood cells and blood-borne particulates, hemosiderin, ceroid, and lipofuscin [12]. The presence of phagolysosome inside macrophage enhances their proficiency to hydrolyze the trapped erythrocyte by proteolytic degradation of hemoglobin. The net result comes with the production of Hem which catabolizes into CO2, biliverdin, and ferrous iron (Fe^{++}). They produced iron that gets its way either to exits from cells or stored inside macrophage in a ferritin form [77]. By Way Of transporter protein "plasma transferrin'', iron can exit from macrophage cell in consequence to bone marrow requirements [54].

Focusing on the ultrastructure of the macrophage cells in our study, it rich with lysosomes, pinocytotic vesicles, and rough endoplasmic reticulum. This can explain being a part of the mononuclear phagocyte system serving a

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significant position in inspecting the blood. The endless journey of hematopoietic cells from the blood into the spleen and back to the blood is an effectual track for these cells to investigate for pathogens and antigens (microorganisms and viruses) using their long cellular processes [7, 43, 54]. These cells produce type I interferons (IFN-α and IFN-β), which carry out an essential position in anti-viral immune responses [54, 78] They participate to immune modulation and preservation of peripheral tolerance [48,79, 80]. It has also been postulated that the MZM (marginal zone macrophage) concluded in linkage and maintenance of lymphocytes (especially B cells) in the marginal zone and their migration into the white pulp, and elimination of cancer cells from circulation [81]. They emit extracellular molecules, proteases, and cytokines that can modify ECM secretion by other cells [82, 83]. Finally, they participate in the regulation of platelet production [84]. The stability marginal zone cells are vital for precise judgment of the immunological outcome on the spleen [20], which highlighted the dependence of these cells on each other to maintain the integrity of the marginal zone [54].

Our last compartment in buffalo spleen is the red pulp. It divided into two main structures: the first one in which the splenic cells organize in cord-like to forlm splenic cords and the other was the place where the blood circulated named splenic sinuses. This matches well with the finding in camel [19, 40], ruminant and porcine [20, 43, 52, 56] and buffalo calf [22]. Meanwhile, splenic sinusoids in sheep and goat perceive in a less abundant and poorly developed form [31, 44].

Our study approaches red pulp in water buffalo spleen using SEM that clarifies the irregular arrangement of splenic cords which associate with a network of reticular cells or interdigitating cells, these cords consisted of lymphocytes, iron crystal-like of hemosiderin pigment, red blood cells, and other splenic cells. Moreover, in between them, we detect small open channels like vessels representing splenic sinuses. The blood retained from these sinuses to the large splenic vein. The thin branch of the trabecular artery shares its end in the red pulp as penciller artery. TEM examination demonstrates the sinusoidal capillaries line with flat endothelial cells and its lumen is occupied by red blood cells. This fits the earlier literature in buffalo calf [22] and sheep and goat [44]. The sinuses were wide and short in the cow. In camel venous sinusoids lines by fusiform to cuboidal fashioned endothelium next to the subscapular cavity [19].

Water buffalo spleen in the current investigation makes up two types of circulation: closed circulation inside the splenic sinuses and open circulation in between the splenic cords. Equivalent results are recorded in the cow by Seki and Abe [51] in the cow. Alshamarry [33] cleared the splenic circulation inside camel red pulp from 2-5 straight branches of penciller artery, into sheathed arterioles, and then into arterial capillaries that open directly into the sinusoids or splenic cords [40,55].

Rebelatto [12] differentiated the closed circulation that the arterial walls are directly connected with the walls of sinuses, opposite to the open circulation, in which arteries terminate blindly along with free blood flow toward splenic cords of the red pulp. Therefore, the blood reaches sinuses via a three-dimensional net of fibroblastic reticular cells and then flows across openings in the walls of the sinuses. From sinuses, the blood runs into veins of the red pulp, from there to trabecular veins to splenic vein [40,41,54, 69,70]. On the other hand, they face antigens through their existence in the area where arterioles drain into blood sinuses permitting them to professionally entrap blood-borne antigen-immune complexes and transport by DCs to marginal zone B cells [75]. This could eventually illustrate the red pulp efficiency in blood filtration through removing foreign material, damaged, and effete erythrocytes by the phagocytosis process, also we can shed the light on this area as storage set for iron, erythrocytes, and platelets [68, 52,53]. Approval of flowing apoptotic cells influences the production of regulatory T cells and antibodies, performing a crucial part in the preservation of peripheral tolerance and modulation of the immune system [79, 80]. Surprisingly, the proficiency of this clearing system is determined by the fact that splenectomy is applied to cure patients with circulating antiplatelet antibodies affecting autoimmune thrombocytopenic purpura [54].

In the red pulp, we concentrate on high endothelial venules (HEVs); by SEM examination HEVs appear like a channel encircled by lymphocytes, a rectangular crystal of iron, and red blood cells. Meanwhile, TEM highlight its content with B and T lymphocytes, and the pericytes are demarcating its external surface. At the ultrastructure level, we spot on the T with a large heterochromatic nucleus, a thin rim of cytoplasm, and few organelles. The iron pack with electron-dense bodies of hemosiderin pigment. We support data mentioned by Miyasaka and Tanaka [85] which illustrated that these venules have specific endothelium that aid to interact with adhesion molecules lying on the surface of the lymphocytes (homing receptors). In contrast to Mebius and Kraal [54], who reported that in the spleen, all cells enter via the marginal zone, unlike other lymphoid organs where high endothelial venules (HEV's) are the site of lymphocyte entry. According to the previous variation, we recommend that high endothelial venules (HEVs) comprise blood vessels specifically designed for the traffic of lymphocytes in some species and absent in others. Since they accumulate in non-lymphoid organs during chronic inflammation driven by infection, allografts, or autoimmunity. More recently, scientists correlate the reduction in tumor size is back to HEVs that possess anti-tumor characteristics. Through which, HEVs engage naive lymphocytes, react

with tumor together with local initiation of cancerous tissue-destroying lymphocytes [86].

In the present literature, we recognize other splenic cells in the red pulp of water buffalo spleens like mast cell, macrophage, natural killer cells, leukocytes, platelets, myofibroblast, and reticular or interdigitating cells. Owing to the spleen is the elimination site to distorted erythrocytes and therefore, it appeals to great reactivity in response to hemiparasitic infections. In Babesia Bovis, the researchers detailed acute change in the spreading of several cells believed to be essential to the spleen-dependent response (leucocyte population included monocytes, macrophages, dendritic cells (DCs) [87], mast cell [63] and large granular natural killer (NK) cells [88]. Therefore, cellular compartments of water buffalo spleen must undergo full examination in diseases, toxicity, and carcinogenicity studies as a potential target site for treatment effects [12].

Mast cells highlight in our study by purple specific granules using a toluidine blue stain. Meanwhile, this cell by TEM encloses centrally located heterochromatic nucleus and electron-dense cytoplasmic granules. In line with previous investigations in other mammals [89]. These granules have an overabundance of preformed and pre-stimulated immunomodulatory composites, involving lysosomal enzymes, biogenic amines, for instance histamine, and proteoglycans. Following activation, mast cells go through degranulation, someplace these preform composites quickly liberated into the extracellular environment [90]. There are also some newly synthesized mediators omitted by activated mast cells, including leukotrienes, prostaglandins, cytokines, chemokines, and growth factors. Hence, we can explain they are critical function, in their ability to modulate various physiological and pathological events through these released compounds. Importantly, some of the released mediators from the granules can cause allergic responses, such as those occurring in asthma and allergic rhinitis [89]. Other researchers propose that the role of the spleen in the development of food allergy is to provide a unique site where antigen-specific T cells induce the development of pathogenic mast cells [91].

On the other hand, in the current research, macrophage cells in the red pulp showed the same structure as in the MZ of water buffalo spleen. In total agreement with the previous records in other mammals [48,54,92] and Gazelles [41]. Authors of this work confirm the scavenger power of red pulp macrophages to eliminate the elderly, impaired platelets, blood-borne substance [43], and senescent erythrocytes, so they protect the organism from overwhelming sepsis [54, 92] and believed to trigger an accumulation of iron release [78]. Iron uptake via RP macrophages can also be vital in restricting the evolution of pathogens by constraining their resource of iron [54].

TEM examination of the natural killer cells (NK) in the red pulp of water buffalo spleen represent by prominent cell receptors, indented heterochromatic nucleus, and abundant cytoplasm that contain lysosomes and ribosomes. It matches with the previous literature in mammals [93- 95]. We supposed data discussed by Maupome et al. [94] that NK cells contribute to important cytolytic cells in innate immunity, a key role in defending hosts against injuries as they represent cytotoxic lymphocytes fighting viral infections and tumors without prior stimulation [95], express inhibitory signals, such as those protecting against lysis of healthy cells [96, 97], kill and lyse infected or transformed cells as well as their capability to cooperate in the creation of suitable immune responses by the aid of immunoregulatory cytokines (e.g., TNF-α and IFNγ) along with chemokines (e.g., CCL3 and CCL4) [98, 99]. Given that, NK highly present in inflamed and cancer tissues [93,100] in line for their shaping in both innate and adaptive immune responses [101].

Bovine natural killer cells respond to a diversity of pathogens that trigger economically critical cattle disorders, involving Mycobacterium Bovis [94, 102, 103], Neospora [104], Babesia [105] and Theileria-infected cells [106]. Hence, we appointed their substantial role in the initiation or delivery of defensive immunological memory and symbolize prospective new targets in vaccination strategies specially for buffalo species [107,108].

At the ultrastructure level of water buffalo spleen, we notice another cell with a bilobed heterochromatic nucleus and electron-dense granules, referred to leukocytes. CCED [109] recorded the same leukocyte type as an eosinophil cell. It represents one of the immune system components. Furthermore, their granules hold various chemical mediators, for example, ribonuclease (RNase), deoxyribonucleases (DNase), eosinophil peroxidase, plasminogen, and lipase [109]. Following their stimulation, eosinophils starring role in the generation and emission of cationic granule proteins [110], reactive oxygen species [111], lipid mediators [112], enzymes, growth factors [113] and cytokines, and tumor necrosis factor (TNF) alpha [110,114]. So, we suggest that they are responsible for combating multicellular parasites [109], viral infections, in fibrin removal during inflammation, allograft rejection, neoplasia, and antigen presentation to T cells [114]. Moreover, eosinophils, in conjunction with basophils and mast cells, epitomize a crucial mediator of allergic reactions, plus asthma pathogenesis as well as the degree of disease severity [115].

The platelet's ultra-structure distinguishes in our work by large numerous alpha granules, small few gamma granules, open canalicular system, and peripheral microtubules with actin filament-forming a marginal bundle. Related results document in ruminant and porcine animals by [48,54,92]. Papenfuss and Cesta [43] return the same result to spleen capacity in the exclusion of malformed platelets from the blood, besides, to be a reservoir

for them. Consequently, the number of platelets may decrease (thrombocytopenia) or increase (thrombocytosis) will reflect certain diseases. Hypersplenism can lead to thrombocytopenia, because of the risen segregating and disruption of platelets in the spleen [56]. We inspire reports that suggest two different mechanisms regarding thrombocytopenia: one conducts by intensifying the activity of changeable pooling of platelets; the further is refereed by phagocytosis process and IgG-coated platelets damage inside splenic tissue, e.g. as noticed in chronic idiopathic thrombocytopenic purpura [116]. After splenectomy, platelets increase in body which reflect the cessation in platelets destruction inside the spleen and uncontrolled regulation through splenic stromal cells and macrophage which in turn will raise the production of them by bone marrow and thrombocytosis yielded [84].

Finally, we notice that the splenic red pulp of water buffalo gets its support by two types of cells: myofibroblast cell and reticular or interdigitating cell. Myofibroblast cell appears by histological examination as a long thin cell with a flat bulged nucleus and a thin rim of cytoplasm. Likewise, by TEM examination, a long cell with a flat heterochromatic nucleus and ill-developed cytoplasm. It is squeezed in between red blood cells. On the contrary, the reticular or interdigitating cell labeled by the large euchromatic nucleus and few ill-developed cytoplasmic organelles. These results come in the line with other documents in other mammals [44, 54], Gazelle [41], ruminant [52], sheep, goat, and camel [22]. These cells not only sharing support but were also the most active cells screening for germs and exogenous substance in the body [49]. Contrary to Polák et al. [57], who mentioned that they do not change to macrophages in physiological conditions, so they do not participate in phagocytosis.

Our study strongly supports that regulatory reticular cells possess its ability to stimulate the differentiation of splenic B cells to a definite subtype of IL-10-emitting regulatory B cells [117]. They also dedicated for antigen presentation to T cells after that induct T cell-dependent immune response [118]. Regarding plasma-blasts, DCs ensure the survival and the modifying ability of these cells to plasma cells inside RP [119]. The dendritic or reticular cells resembling smooth-muscle cells emerge outfitted by a plenty of thin filaments combined with their plasmalemmal dense bodies [50]. In view with pervious literature and our results, we can reflect buffalo reticular cells in red pulp to myofibroblasts cells that can join in splenic contraction as well as splenic fibrogenesis [12].

V. CONCLUSION

Spleen assembly in a way that ensures precise blood monitoring and immune stability. We shed light on the normal splenic structure of water buffalo, in terms of their anatomical, histological, histochemical, and ultrastructural features. We linked our results with their associated functions. Consequently, when immunologists and pathologists apply these techniques, can easily differentiate, diagnose, and regulate the potential impact of various buffalo diseases.

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