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MICROANATOMICAL STUDIES ON THE SPLEEN'S CAPSULE IN ONE HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

The aim of the study was to describe histological structure of camel spleen. The present study was conducted on 6 spleens of camel (irrespective of sex and age) procured from the apparently healthy euthanased/ dead camels (irrespective of sex and age). The spleen was covered by moderately thick fibro-elastic and muscular capsule invested by the serous peritoneal covering. The present study has shown that the capsule of the camel spleen is divided into outer connective tissue and inner smooth muscle layers constituting about 1/3 and 2/3 of the capsule thickness. Vascular and avascular trabeculae extended from the capsule, containing arteries and nerves without any trabecular veins, the latter being divided structurally into primary and secondary trabeculae. Subcapsular and peri-trabecular blood sinuses around primary and vascular trabeculae were found unique to the camel spleen.

Key words: Camel, blood analysis, haematology, lactation, physiology

Spleen is more complex than other lymphoid tissues and is an important reservoir of blood (Melvin and William, 1996). According to Bloom and Fawcett (1975), animals with a large blood volume (Equine, Ruminants and Carnivores) have scanty white pulp and a robust connective tissue. Yagil (1985) suggested that desert species of animals have larger spleen than the animals in temperate climate. Radmehr (1997) described vascular segments in the spleen of one humped camel.

The spleen is the largest organ of the lymphoid system (Smuts and Bezuidenhout, 1987) and plays an important role in immunological defence (Das *et al,* 2005). The spleen has a semi-lunar shape in camel, with a rounded dorsal extremity, separated from the main body by a narrow zone (Aichouni, 2008).

The gross, microscopic and ultrastructural details of the spleen of camel has been studied previously (Hayfaa, 2010; Abd El Aal, 1994). The histology of capsule of spleen is least studied.

The objective of present study is therefore, to describe the histological structure of capsule of dromedary spleen to understand its functionality and difference from other species.

Materials and Methods

IAEC (Institutional Animal Ethics Committee) approval:

Present investigation was approved by IAEC of CVAS, Bikaner as per CPCSEA norms vide order No. CVAS/IAEC/2017/06 dated 30/11/2017.

The present study was conducted on 6 spleens of dromedary camels (irrespective of sex and age). The cadaver spleen were obtained from the apparently healthy euthanased/dead camels (irrespective of sex and age) from Veterinary Clinical Complex, CVAS, Bikaner.

Light microscopical studies of the capsule of spleen was done to study:-

a) Distribution and types of connective tissue fibres.

b) Distribution of muscle fibres.

c) Observing the details of splenic tissues and cells.

The tissues (2mm size) were collected from different anatomical regions of spleen and preserved in 10% formal saline, Bouin's fluid and Zenker's fluid for 48hrs, 15hrs and 18hrs, respectively. These were processed for light microscopy by using paraffin

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of melting point 58-60°C. The paraffin blocks were sectioned to obtain 5-6µm thick sections which were subjected to the following routine histological stains to demonstrate different components of spleen.

a) Eharlich's Haematoxylin and Eosin stain for routine observation (Singh and Sulochana, 1997).
b) Gomori's method for reticulum (Luna, 1968).
c) Verhoeff's elastin stain for connective tissue fibres (Singh and Sulochana, 1997).
d) Van Gieson stain for collagen fibres (Singh and Sulochana, 1997).
e) Masson's trichrome method for collagen fibres (Singh and Sulochana, 1997).
f) Crossman's Modification of Mallory's Triple stain elastic and collagen fibres (Singh and Sulochana, 1997).
g) Gomori's method for Iron (Luna, 1968)

Results

Capsule

The spleen was covered by moderately thick fibro-elastic and muscular capsule invested by the serous peritoneal covering. Serous peritoneal layer covered the outer most layer of capsule made up of the mesothelial cells having squamous to cuboidal cells. The capsule was divided into clearly demarcated outer and inner layers (Fig 1). The cytoplasm was eosinophilic and very scanty and only the lightly basophilic nuclei were visible. The outer layer consisted mainly of connective tissue including collagen, elastic and reticular fibres with few smooth muscle cells. The inner layer was composed predominantly of smooth muscle cells supported by reticular, collagen and elastic fibres. The rich smooth muscle fibres in the capsule were arranged in three layers. An outer layer of smooth muscle fibres was arranged longitudinally parallel to the surface adjacent to the connective tissue present just below the mesothelium (Figs 2, 3, 4, 5 and 7). The middle layer fibres were arranged obliquely or transversely. The middle layer was the widest as compared to the other two layers. The muscle fibres were interwoven with the collagenous and elastic fibres (Figs 3 and 4). The reticular fibres were uniformly arranged throughout the capsule. The framework of the capsule presented mixed population of reticular, collagen and elastic fibres (Figs 2 and 3). The collagen fibres were densely arranged along with few reticular fibres just above the outer layer of the smooth muscle fibres (Fig 4). The elastic and reticular fibres were prominent in the inner most zone. A uniform layer of the reticular fibres was present being arranged longitudinally in the inner most part of the capsule. Vertically oriented fibres continued the framework of trabeculae, as compared to the fibres present in the capsule.

Trabeculae

Branching connective tissue trabeculae emerged from the innermost zone of capsule and entered into the interior of the splenic parenchyma and subdivided into smaller compartments forming a net like framework (Fig 1, 2, 5, 6). Trabeculae were uniquely divided into vascular and avascular trabeculae. The vascular trabeculae contained arteries and nerves but no veins (Fig 6). The avascular outnumbered the vascular trabeculae and were divided into primary and secondary trabeculae (Fig 6). The primary trabeculae originated from the capsule and had a similar structure to that of the inner layer of the capsule, being composed mainly of smooth muscle cells lying parallel to the longitudinal axis of the trabeculae and supported by reticular, collagen and elastic fibres. The secondary trabeculae were composed mainly of parallel smooth muscle cells with reticular fibres among them (Fig 3, 5, 6).

The large trabeculae contained arteries, veins and nerve fibres, which were surrounded by smooth muscle fibres (Fig 3, 6). The connective tissue fibres, i.e. elastic, reticular and smooth muscle fibres continued vertically from the capsule and were arranged along the longitudinal axis of the trabeculae (Fig 4, 6). The tightly packed smooth muscle fibres were oriented along its longitudinal axis (Fig 5). A wide meshwork was formed by the thin reticular fibres along with few collagenous fibres. The reticular fibres become progressively thinner in the terminal branches of the trabeculae. The elastic fibres of varying concentrations were oriented in different planes in the trabeculae (Fig 6).

Discussion

Capsule

The present study has shown that the capsule of the camel spleen is characteristically thick and divided into outer connective tissue and inner smooth muscle layers constituting about 1/3 and 2/3 of the capsule thickness, respectively. Findings of the present investigation were in accordance of Hegazi (1953), Abd El Aal (1994), Zidan *et al* (2000), Maina *et al* (2014), Bello *et al* (2016) and Alhaji *et al* (2019).

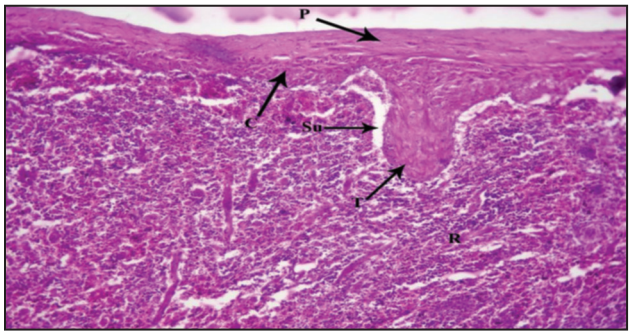

Fig 1. Section of the spleen showing outer and inner layers of capsule and peritoneal covering. C-Capsule, P-Peritoneum, T-Trabeculae, R-Red pulp, Su- Splenic sinusoid. (H&E stain, 100X).

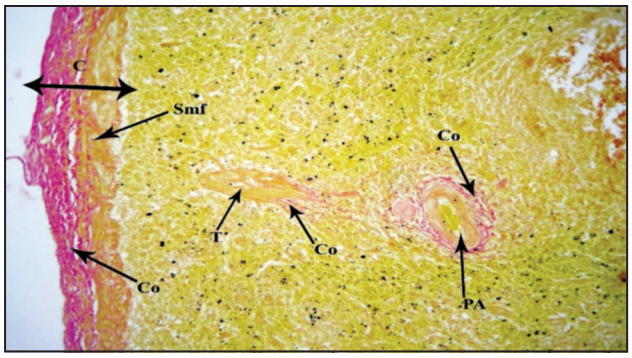

Fig 3. Section of spleen showing muscle fibres interwoven with the collagenous and elastic fibres in capsule and trabeculae. C- Capsule, Smf- Smooth muscle fibres, Co- Collagenous fibres, PA- Pulp artery, T- Trabeculae. (Van Gieson stain 40X).

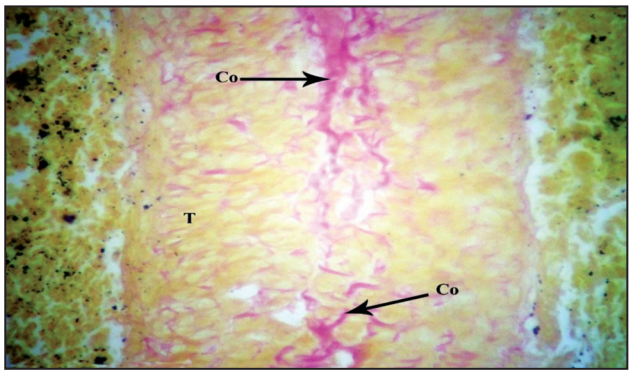

Fig 5. Section of spleen showing tightly packed smooth muscle fibres oriented along its longitudinal axis forming a wide meshwork by the thin reticular fibres along with few collagenous fibres. T- Trabeculae, Co- Collagenous fibres (Van Geison stain 400X).

The capsule was invested by serous peritoneal covering consisted of simple squamous mesothelial cells, which was in accordance with the observation of Bashir and Bernard (2015). Simple squamous mesothelial cells of peritoneal covering were irregular in shape with centrally placed spherical nucleus and attenuated strands of cytoplasm were observed in the present study.

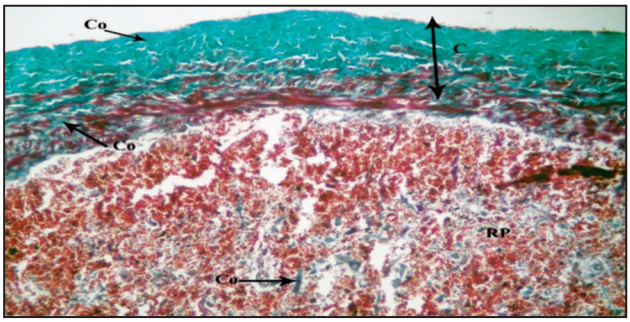

Fig 2. Section of spleen showing outer layer of capsule having collagenous fibres and collagenous fibres in RP- Red pulp. (Masson's Trichrome stain 100X).

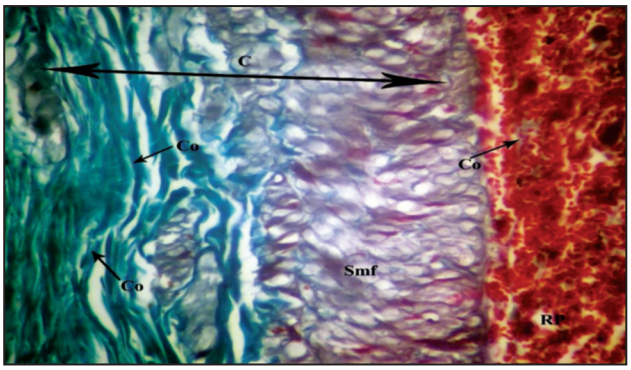

Fig 4. Section of spleen showing inner layer of capsule composed predominantly of smooth muscle cells supported by reticular, collagen and elastic fibres and collagen fibres were densely arranged along with few reticular fibres just above the outer layer of the smooth muscle fibres. C- Capsule and RP- Red pulp. Smf- Smooth muscle fibre, Co- Collagenous fibre. (Masson's Trichrome stain 400X).

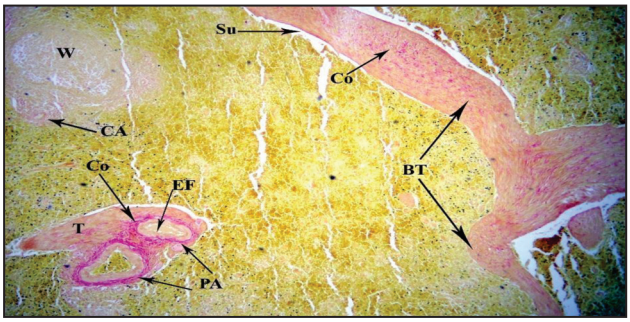

Fig 6. Section of spleen showing branching connective tissue trabeculae entered into the interior of the splenic parenchyma having vascular and avascular trabeculae. Vascular trabeculae contained arteries and nerves but no veins. Co- Collagenous fibres, EF- Elastic fibres, T- Trabeculae, W- White pulp, PA- Pulp artery, CA- Central artery, Su- Sinusoid, BT- Branch of trabeculae (Verhoeff's Elastic stain 40X).

The smooth muscle fibres were interwoven with collagenous and elastic fibres. The reticular fibres were uniformly arranged throughout the capsule as also described by Awal *et al* (1992) in indigenous cattle, Devi (2012) in Marwari goat and Jadhav *et al* (2019) in domestic pig.

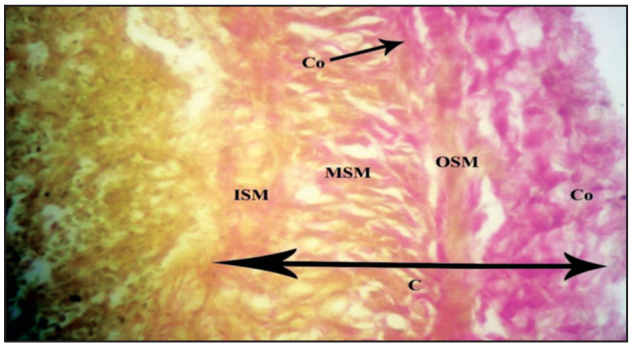

Fig 7. Section of spleen showing rich smooth muscle fibres in the inner layer of capsule arranged in three layers; outer, middle and inner. The smooth muscle fibres are arranged parallel to the surface in the outer and inner layer and oblique in the widest middle layer. T collagenous fibres in capsule, Co- Collagenous fibres, OSM- Outer layer of smooth muscle fibres, MSM- Middle layer of smooth muscle fibres, ISM- Inner layer of smooth muscle fibres, C- Capsule. (Verhoeff's Elastic stain 400X).

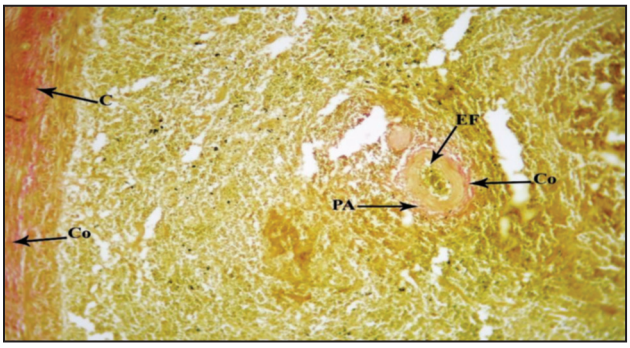

Fig 8. Section of spleen showing collagenous fibres present in capsule, artery and pulp along with some elastic fibres. Co- Collagenous fibres, PA- Pulp artery, EF- Elastic fibres, C- Capsule (Verhoeff's Elastic stain 100X).

The smooth muscle fibres were arranged in three layers; outer, middle and inner. The fibres were parallel to the surface in outer and inner layer and oblique in middle layer. These findings were in consonance with the findings of Dellmann and Brown (1987) in pig, Bajpai (1992) in goat, Thanvi (2002) in sheep and Devi (2012) in Marwari goat. The significant elastic component of the connective tissue fibres observed with trichrome stain may account for high elasticity of the spleen (Hayfaa, 2010).

The thickness of the capsule, trabeculae and concentration of smooth muscles are very important agents to make strong contraction when the body need the blood and the smooth muscle concentration may play a role in the immune reactions, and this agree with what was found by Pinkus *et al* (1986) in their study on human spleen. The arrangement of the smooth muscles in different layers in the capsule assisted smooth muscle in the trabeculae thereby contracting the spleen and to pump out the excess blood in to the circulation at the time of emergency. This simulated the finding of Banks (1981) in domestic animals that elastic fibres allowed large volume changes, whereas the contractions of smooth muscles fibres discharged the blood from the organ.

Trabeculae

The present investigation findings about trabeculae concurred, with the findings of McLeod *et al* (1964) in bovine, Raghavan (1964) in ox, Getty (1975) in horse and Maina *et al* (2014) and Alhaji *et al* (2019) in camel.

Two to three branches of the trabeculae were observed in the present study. The large trabeculae contained arteries, veins and nerve fibres, which were surrounded by smooth muscles. These findings were similar to the findings of Dellmann and Brown (1987) in pig and ruminants, Trautmann and Fiebiger (1957), Nickel *et al* (1979), Devi (2012) in Marwari goat, Gnanadevi *et al* (2019) in sheep and goat and Rahmoun *et al* (2019) in rabbit spleen.

Conflicts of Interest

The authors declare no conflict of interest.

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