Histochemical Studies on the Camel Extraocular Muscles and Their Innervation

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Abstract

The current study was conducted to examine the one-humped camel (Camillus dromedarius) extraocular muscles and their innervation. Furthermore, the S-100 immuno-expression within these muscle fibers was analyzed. Fourteen eyeballs, from native Egyptian camels (of both sex), were collected. Processing and preparation of the obtained tissue samples took place after being immersed in 10% neutral buffered formalin. After that, they sectioned, and then subjected to the following stains; Hematoxylin & Eosin (H&E) for determination of muscle spindle, nerve trunk, and Golgi tendon organ, PAS and Alciane blue for detection of mucopolysaccharides, and silver stain to examine muscle fibers and nerve trunk affinity. The obtained results showed that the extraocular muscles consisted of seven muscles: four recti, two obliques, and one retractor bulbi muscle. Each muscle had a muscle spindle that was encapsulated with a connective tissue capsule with intrafusal muscle and paraxial space. As well as, the Golgi tendon organ was found at the musculotendinous junction. Immuno-expression of the seven muscles showed varying degrees of immunostaining affinity to the S-100 protein.

Key words: Histochemistry, S-100, Camel, Extraocular Muscles, Innervation

Introduction

The one-humped camel (*camelus dromedarius*) occupies the north and south area of latitude 15ºC which is characterized by being an arid or semiarid zone ***(Wilson, 1984***). To survive in the harsh environmental conditions, the camel managed to adapt itself physiologically and anatomically ***(Yagil, 1984).*** This is well expressed by temperature regulation, concentration of urine, soft feet, long limbs and small well – protected eyes. This animal has sharp eyesight and some distinctive ocular anatomical features ***(Yadegari et al., 2013).*** The gross anatomy of the eye has been extensively described in equines ***(Sisson and Grossman, 1975),*** bovines ***(Dyce and Wensing, 1971),*** sheep ***(May., 1954)*** and goat

(Constantinescu, 2001). The basic architecture of the eye was similar in all farm animals except to minor differences. The extraocular muscles contract much more quickly than other voluntary muscle ***(Cooper, 1930).*** The great speed of contraction of extraocular muscles is in keeping with the regulation of saccadic eye movement. Regardless of the socioeconomic importance of the one-humped camel, the histological structure of the eye has been received a little attention. From the available literatures, very few studies have demonstrated the histochemical & immunoreaction of the extraocular muscles of the camel eye. Therefore, the aim of this work is to clarify the histological structure and histochemical reaction as well as S-100 protein immunoreaction of the extraocular muscles of the one-humped camel eye.

Materials and Methods

I- Sample collection and the histological procedures.

This study was carried out on fourteen eyes of the adult one-humped camel (*camelus dromedarius)*collected from Ismailia Slaughter house. The study protocol was ethically approved by Research Ethics Committee at Faculty of Veterinary Medicine, Suez Canal University, Egypt (Approval No. 2.18.15)

The following seven extraocular muscles (the right and left eyes) were dissected: dorsal rectus, ventral rectus, lateral rectus, medial rectus, dorsal oblique, ventral oblique, and retractor bulbi muscle. The afore-mentioned muscles were fixed in 10% neutral buffered formalin. They were subjected to routine histological procedures, dehydrated, cleared, and embedded in paraffin wax. Then, they were sectioned serially at 6-7 µm in thickness.

The paraffin sections were stained with the following stains: -

1. **Harris's hematoxylin & Eosin (H&E)**: for studying the general histological structure of the muscular tissue.
2. **Masson's Trichrome stain**: for studying the collagenic bundles.
3. **Alcian blue (PH 2.5) stain**: for detection of acidic mucopolysaccharides.
4. **Periodic acid Schiff (PAS) technique**: for detection of neutral mucopolysaccharides.
5. **Best`s Carmine stain**: for detection of glycogen.
6. **Bielschawsky, s Silver stain**: for detection of nerve fibers.

II- Immunohistochemical procedures

The collected tissue samples of the muscles were fixed in 4 % formaldehyde at pH (7.4) for 2 days; then processed and mounted on positively charged slides for immunohistochemical detection of S-100 protein. All procedures were done according to Vectastain Elite ABC kit (Vector laboratories. California, USA).

Polyclonal S-100 Protein rabbit pAb (Catalog number A14780, Abclonal Scientific, Co., Swiss prot) was used

as a primary antibody. Slides were incubated in 3,3´-diaminobenzidine peroxidase enzyme substrate (DAB) for 30 min. and counterstained with Mayer´s hematoxylin; they were dehydrated, cleared, and mounted with DPX. For negative control, Sections were treated with similar steps with exception of the primary antibody.

III- Measurement of immunostaining intensity of S-100 Protein.

For detection of the percentage area % of immunostaining intensity of S-100 protein in extraocular muscles (intrafusal fibers); images of the selected parts were analyzed using the image J software (at scale bar 50µm). The histological and histochemical procedures were followed as outlined by ***(Carson, 1997).***

Results

The four recti muscle; dorsal, ventral, medial and lateral as well as two oblique muscles; dorsal and ventral and retractor oculi muscle were the sum of seven extraocular muscles of the one-humped camel eye (Fig. 1A).

Histological findings of the dorsal, ventral, lateral & medial recti muscles

Sections of the four recti muscles stained with H&E showed that the muscle fibers were arranged in varying diameters and lengths separated by connective tissue fibers (Fig. 1B).

The muscle spindles were surrounded by thick capsule of collagen fibers, which enclosed well developed paraxial space, intrafusal muscle fibers of varying sizes (Fig. 2A). Muscle spindles were less in number in the ventral recti than the dorsal one and their nuclei were more peripheral with few numbers of the intrafusal muscle fibers (Fig. 2B). In the lateral one, the muscle spindle had fusiform shape and a wide paraxial space. Different sizes of intrafusal muscle fibers were recorded (Fig. 2C), meanwhile, the muscle spindle of the medial one was spherical and containing variable sizes of intrafusal muscles with a wide paraxial space containing collagenic fibers (Fig. 2D). Dilated blood vessels and nerve trunk were seen neighboring the muscle spindles.

The Golgi tendon organ of the four recti muscles was fusiform in shape & surrounded by C.T capsule (Fig. 3A); wedged among the collagen & skeletal muscle bundles (Fig. 3B).

In dorsal, lateral, and medial recti muscle; a strong PAS reaction was evident in the Golgi tendon organ (Fig. 4A), nerve trunk, blood vessels and skeletal muscle fibers. In the ventral recti muscle, a moderate reaction was demonstrated (Fig. 4B). Alcian blue reactivity was intense in the ventral, lateral and medial recti muscles (Fig. 4C), meanwhile; it was faint in the dorsal one (Fig.4D). A week to moderate glycogen deposition in the muscle fibers was observed in the four recti muscles

(Fig. 4E). Examination of the recti muscles stained by silver stain showing a strong reaction in the nerve fibers which arising from the nerve trunk to innervate the skeletal muscle fibers (Fig. 4F).

Immunohistochemical detection of the dorsal rectus muscle proved an intense immunoreactivity of S-100 protein in the cytoplasm and nuclei of the muscle fibers and nerve trunk fibers. A strong reaction was obvious in the muscle spindle and intrafusal muscle, in the nerve trunk (Fig. 7A).

Histological examination of retractor oculi muscle

A varying diameters of muscle fibers with varying lengths was demonstrated, A longitudinal oriented nerve trunk in between the muscle fibers (Fig. 5A). Muscle spindle was larger in size and enclosed by a connective tissue capsule containing nerve trunk, blood vessels, the paraxial space and intrafusal muscle fibers (Fig. 5B). The intensity of PAS was ranged from strong to moderate reaction of skeletal muscle fibers (Fig. 5C). As well as, strong Alcian blue reaction - (Fig. 5D) and moderate glycogen content of skeletal muscle fibers and nerve trunk was observed. (Fig. 5E). Silver stain showed strong reaction in the nerve fibers which arising from the nerve trunk to innervate the muscle fibers. (Fig. 5F). Immunohistochemical detection of the retractor oculi muscle proved a strong immune- reactivity of S-100 in both longitudinal and cross sections of muscle fibers. A recorded negative reaction of the blood vessels was observed. (Fig. 7B).

Histological observation of dorsal and ventral oblique muscles

The muscle fibers of the dorsal & ventral oblique muscles were arranged in varying directions; longitudinally, oblique, and transversely intermingled with C.T bundles, nerve trunk and blood vessels (Figs.6A&6C). A fusiform muscle spindle having intrafusal muscle fibers of variable diameter, paraxial space was detected. The extrafusal muscle fibers were obliquely directed with peripheral nucleus (Fig.6A). Golgi tendon organ was demonstrated among the C.T bundles; specially at the musculotendons junction (Fig.6B). The Golgi tendon organ, muscle fibers and the muscle spindle, nerve trunk were strongly reacted to PAS (Fig. 6B). A strong Alcian blue reaction of acid mucosubstances was observed especially in skeletal muscle fibers (Fig. 6D). In addition, Sections showed moderate glycogen content of the skeletal muscle fibers (Fig. 6E).

Silver stain reaction was intense in the nerve fibers which arising from the nerve trunk to innervate the muscle fibers. (Fig.6F). Immunohistochemical detection proved a strong immune- reactivity of S-100 protein in the intrafusal muscle of the muscle spindle and nerve trunk (Fig. 7C & D).

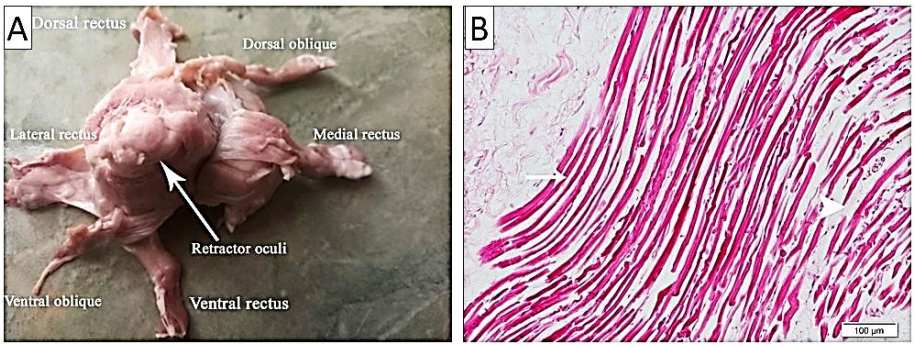

Fig. (1A) A photomicrograph showing seven extraocular muscles of camel eye.
Fig. (1B) A photomicrograph of dorsal rectus muscle showing different diameters (arrow) and lengths (arrowhead). H&E.

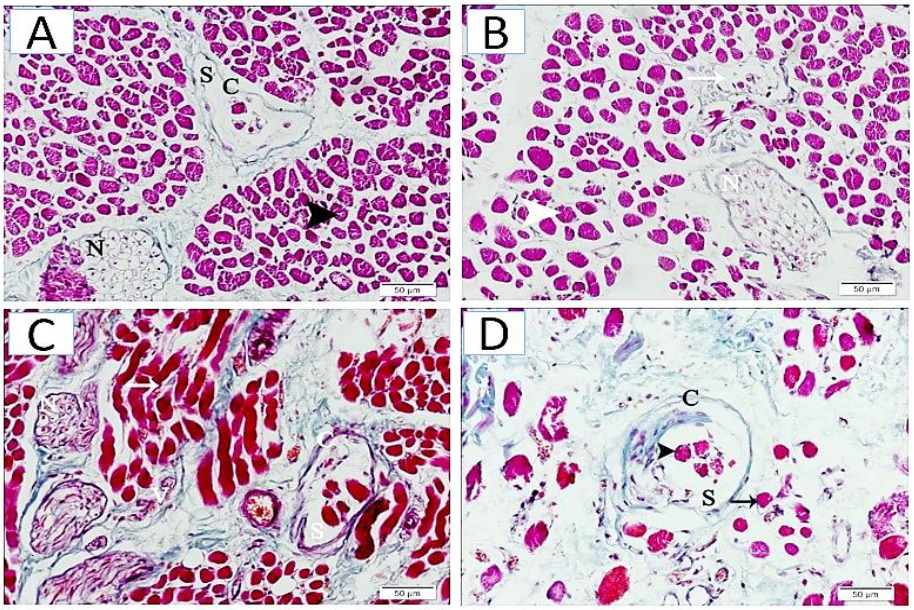

Fig. (2) A photomicrograph of the four recti muscles
(2A) The dorsal rectus muscle showing muscle spindle, capsule (C) periaxial (S) and intrafusal muscle fibers, nerve trunk (N). Masson's Trichrome.
(2B) The ventral rectus muscle showing muscle spindle (arrow), nerve trunk (N), skeletal muscle fibers (arrowhead). Masson's Trichrome
(2C) The lateral rectus muscle showing muscle spindle surrounded by C.T capsule (C) and periaxial space (S). Note the nerve trunk (N), blood vessels (V) and muscle bundles (arrow). Masson's Trichrome.
(2D) The medial rectus muscle showing muscle spindle, capsule (C), periaxial(S), intrafusal muscle (arrowhead) and extrafusal muscle fibers (arrow) Masson's Trichrome.

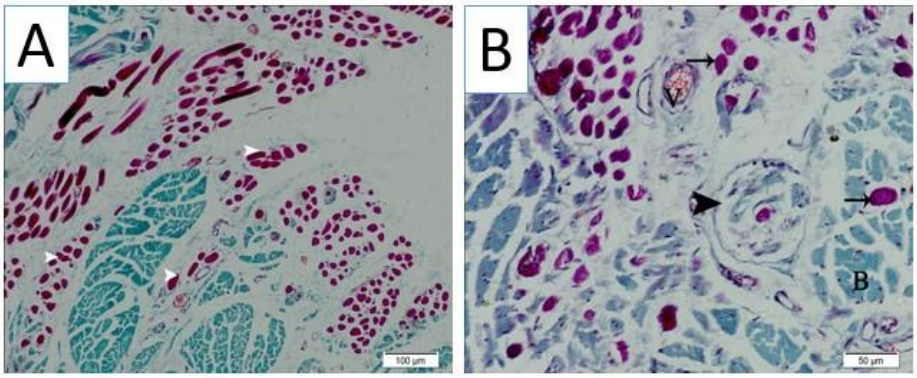

Fig. (3) Photomicrographs of Golgi tendon organ

Fig. (3A) The lateral rectus muscle showing different directions of muscle fibers & Golgi tendon organ (arrowhead). Masson's Trichrome**.**

Fig. (3B) The medial rectus muscle showing Golgi tendon organ (arrow) muscle spindle (arrowhead) & collagen bundles (B). Masson's Trichrome.

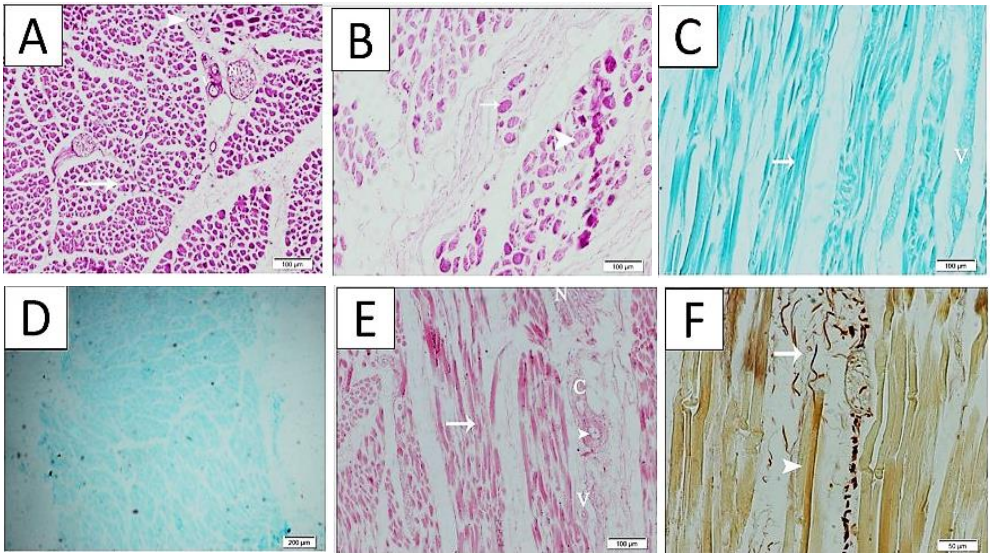

Fig. (4) Photomicrographs of recti muscles

(4A) The dorsal rectus muscle showing strong PAS reaction in the muscle fibers (arrow), interfacial muscle of the muscle spindle (arrowhead), & nerve trunk (N). PAS.

(4B) The ventral rectus muscle showing PAS moderate reaction in the skeletal muscle fibers (arrowhead), intrafusal muscle (arrow). PAS**.**

(4B) The ventral rectus muscle showing PAS moderate reaction in the skeletal muscle fibers (arrowhead), intrafusal muscle (arrow). PAS**.**

(4C) The lateral rectus muscle showing strong Alcianophilic reaction in the skeletal muscle fibers (arrow) & blood vessels. Alcian blue (PH 2.5)

(3D) The dorsal rectus muscle showing faint Alcianophilic reaction. Alcian blue (PH 2.5)

(4E) The lateral rectus muscle showing moderate glycogen deposition in the skeletal muscle fibers (arrow), capsule (c) intrafusal muscle (arrowhead), nerve trunk (N) and blood vessels (b). Best's Carmine.

(3F) The ventral rectus muscle showing nerve fibers (arrow) arising from nerve trunk (N) that innervate in skeletal muscle fibers (arrowhead). Silver

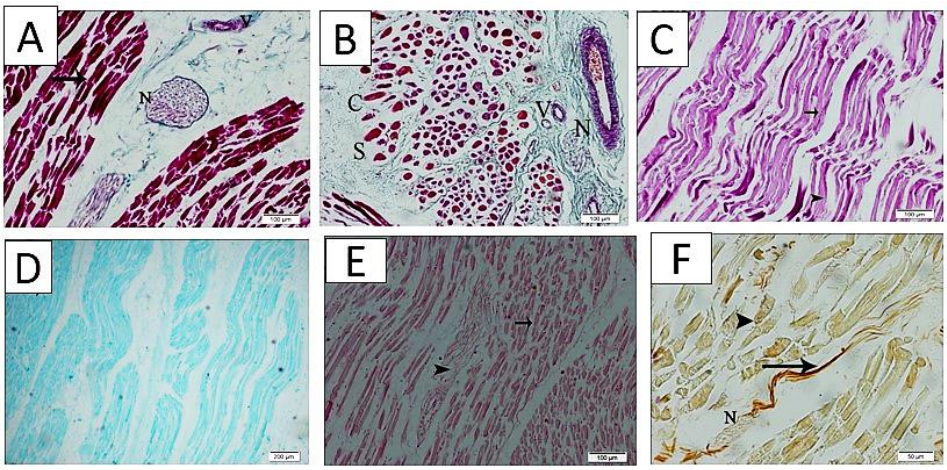

Fig. (5) Photomicrographs of retractor oculi muscle.

(5A) Showing longitudinal muscle fibers (arrow), nerve trunk (N) and blood vessels (V). Masson's Trichrome**.**

(5B) Showing muscle spindle, capsule (C), periaxial space (S), intrafusal muscle fibers (arrow), Golgi tendon organ (arrowhead), blood vessels (V) & nerve trunk (N). Masson's Trichrome.

(5C) Showing a variable reaction to PAS; strong in some skeletal muscle fibers (arrow) and moderate in other ones (arrowhead). PAS.

(5D) Showing Alcianophilic reaction of skeletal muscle fibers (arrow). Alcian blue (PH 2.5).

(5E) Showing moderate glycogen content of the skeletal muscle fibers (arrow) and in the nerve trunk (arrowhead). Best's Carmine.

(5F) Showing reaction in the nerve fibers (arrow) arising from nerve trunk (N) to innervate the muscle fibers (arrowhead). Silver stain.

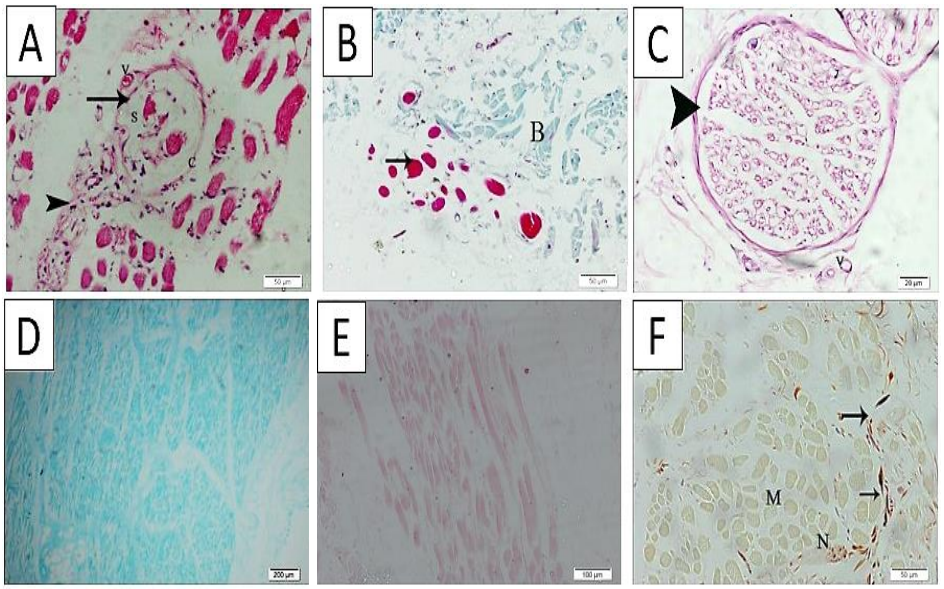

Fig. (6) Photomicrographs of dorsal & ventral oblique muscle.
(6A) The ventral oblique muscle showing muscle spindle (arrow), capsule (C), periaxial space (S) & intrafusal chain fibers (arrowhead). Note blood vessels (V). H&E
(6B) The dorsal oblique muscle showing Golgi tendon organ (arrow) and collagen bundle (B). Masson's Trichrome.
(6C) The nerve trunk showing PAS reaction in axons, endoneurium and perineurium connective tissue (arrowhead), blood vessels (V). PAS
(6D) The dorsal oblique muscle showing moderate Alcianophilic reaction. Alcian blue (PH 2.5)
(6E) The dorsal oblique muscle showing a moderate glycogen deposition in the muscle fibers. Best's Carmine.
(6F) The dorsal oblique muscle showing intense silver stain reaction of the nerve fibers (arrow), nerve trunk (N) with faint reaction of muscle fibers (M). Silver

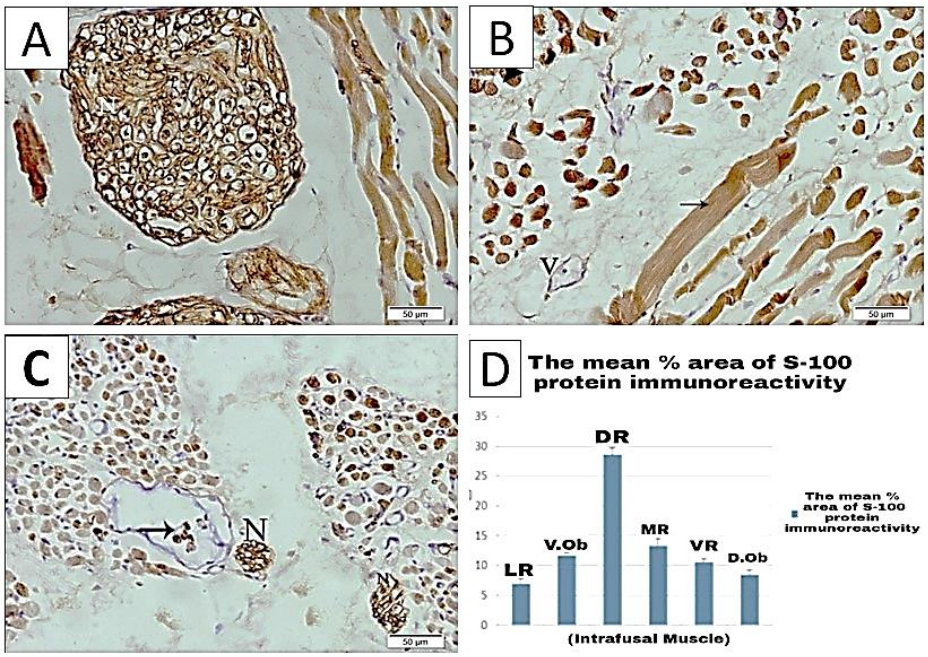

Fig. (7) Photomicrographs of the immunoreactivity of S-protein
(7A) The dorsal rectus muscle showing intense immune- reactivity of S-100 protein of muscle fibers and nerve trunk (N). S-100.
(7B) The retractor oculi muscle showing strong immune reactivity of S-100 protein in muscle fibers (arrow). Note a negative reaction of the blood vessels (V). S-100.
(7C) The ventral oblique muscle showing strong immune reactivity of S-100 protein in intrafusal muscle fibers of the muscle spindle (arrow) & nerve trunk (N). S-100**.**
(7D) The mean % area of S-100 protein intensity of intrafusal muscle fibers of the four recti and two oblique extraocular muscles. Note the highest intensity was in the dorsal rectus muscle. All values were expressed as mean ± S.E.M. using one-way ANOVA followed by the Bonferroni's test for multiple comparisons.

Discussion
The ocular muscle of the camel had the same distribution of skeletal muscles as in tongue, this may reflect the importance of this distribution to fulfill the need for the movement of the eye easily and in many directions ***(Eidaroos, 1991)*** in camel. In this study, the ocular muscles were poor in glycogen, this manifested by a continuous movement of these muscles, which leading to glycogenolysis and production of energy. This might be the main cause of the poverty of this

muscle in glycogen ***(Eidaroos, 1991)*** in extraocular muscles.

In silver-stained sections, variable thickness and length of nerve fibers muscles was recorded, this result was in coincidence with ***Ibrahim (2016)*** in camel.

In this study, the camel extraocular muscles had muscle spindles; they were designed to sense the length of the muscle fibers during contraction, while tendon organs were concerned with the forces developed by the fibers **(McComas, 1996).** Many studies were described the presence of muscle spindles in extra ocular muscles like camel ***(Fath El-Bab et al., 1982) and (Abuel-Atta et al., 1997),*** Sheep ***(Bulmer et al., 2000),*** goat **(*Farag, 2008*).** In contrast, muscle spindles were absent; as in dog, cat, rabbit, horse, domestic pig, and macaque monkey, ***(Huber, 1989; Fukuda, 1985; Billig, Delmas and Buisseret, 1997)*.** Muscle spindles had different shapes and each one was enclosed by a capsule material which could be demonstrated by staining with H&E. This substance was PAS and Alcian blue positive, which might be indication of mucous in nature (acidic mucopolysaccharides).

Through this work, encapsulated structures containing intrafusal muscle fibers were seen could not be considered nuclear bags or chain fibers as their nuclei were found at the periphery not in the center of the muscle fiber. These intrafusal muscle fibers were like extradural muscle fibers but they were thinner. which arising from the nerve trunk to supply the ocular

that contained intrafusal muscle fibers. This result was agreed with ***Blumer et al. (2003)*** in cow and ***Farag (2008)*** in goat. The importance of the capsule was to maintain the integrity of the internal microenvironment of the spindle ***(Dow, Shinn and Ovalle, 1980)*** and ***(Fukami 1992).*** Also, it had a putative role in providing a regeneration for intrafusal fibers. ***(Rogers and Carlson, 1981).***

A large paraxial space separated the intrafusal muscle fibers from the capsule. This result was in accordance with ***Fath El-bab et al., (1982)*** in camel. In opposing, ***Ruskell (1989)*** was demonstrated the limited or absent paraxial space in human. The fluid filled space of paraxial space was contained fine acidophilic

Meanwhile. These were in one line with ***Fredrich et al. (2007).***

In this study, Golgi tendon organs were located at the junction of muscles with their tendons. This result was on the line with the statement of ***McComas (1996) and Farag (2008)*** in goat. Meanwhile, in cat, rabbit, and guinea pig; the Golgi tendon organs in extraocular muscles were absent ***(Huber, 1989, Maier et al., 1974*** and ***Billig et al., 1997).*** The paraxial space of the Golgi tendon organs in goat

contained mucopolysaccharides rich fluid ***(Ruskell, 1990)***

This study showed that, S-100 protein immunostaining affinity was most intense in muscle fibers in most of extraocular muscles and intrafusal muscle as well as in nerve trunk, the muscle fibers showed a slight reaction, in the blood vessels a negative reaction were reported. These results were not mentioned in the available literatures was observed in blood vessels which described the camel extraocular muscles. Immunohistochemically analysis of the cellular distribution of S-100 protein in adult skeletal muscles revealed that S-100 α staining was associated with muscle cells, while S-100β staining was associated with non-muscle cell ***(Danna, 1991).*** S-100 had many functions in cell-cell adhesion protein synthesis and energy metabolism excitation contraction and structure of biochemistry of the thick and thin filaments in skeletal muscle. S-100 protein has been reported to regulate protein kinase activity ***(Kuo et al., 1986a).***

Also, S -100 protein had detected in peripheral nerve cells. It was possible that S-100 positive cells in fast-twitch fibers were muscle cell, whileS-100 and staining was observed throughout the slow twitch muscle fiber cells **(Sugimara et al.,1989)**. Other author suggested that, S-100 proteins are calcium modulated proteins & act by regulating the activity of the other proteins ***(Doneto and giambanco, 1989).*** Moreover, ***Salibian and Montalti (2009)*** reveled that S -100 protein was calcium activated signaling protein, The S-100 protein interact with other protein to make biological function such as cell migration, growth, proliferation, differentiation, apoptosis, contraction.

Conclusion

We could conclude that, the extraocular muscles of the camel eyes and their innervation accommodated the environmental condition; this was manifested by the different diameters and lengths of the extraocular muscles, to fulfill the need for the movement of the eye easily. The ocular muscles were deficient in glycogen, this might be owing to the continuous withdrawal of glycogen for movement. They had numerous muscle spindles which designed to sense the length of the muscle fibers during contraction. Also, S -100 protein immunostaining was detected throughout the ocular musculature. We suggest extra studies on the ultrastructure of ocular muscles of the camel eye.

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