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NM Rajashailesha
Associate Professor, Department of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

RV Prasad
Retd Professor, Veterinary College, Hebbal, Bengaluru, Karnataka, India

Arun A Sha
Director-Research and Veterinary Operations Wildlife SOS, Bannerghatta Bear Rescue Center, Bannerghatta Biologica Park, Bengaluru, Karnataka, India

KV Jamuna
Retd Professor, Department of Veterinary Anatomy, Veterinary College, Hebbal, Bengaluru, Karnataka, India

ML Satyanarayana
Retd Professor, Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru, Karnataka, India

S Ganga Naik
Professor and Head, Department of Veterinary Anatomy, Veterinary College, Gadag, Karnataka, India

Corresponding Author
NM Rajashailesha
Associate Professor, Department of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

Gross morphology and histology of extraocular muscles in sloth bear (*Melursus ursinus*)

NM Rajashailesha, RV Prasad, Arun A Sha, KV Jamuna, ML Satyanarayana and S Ganga Naik

Abstract
The extra-ocular muscles of are accessory organs of the eye (*Organa oculi accessoria*) which are involved in ocular movement and are located between the sclera and the wall of the orbit. These muscles are classically classified as four rectus, two oblique and one retractor bulbi. Though, their details and potential associations with sloth bear behaviour are limited. The aim of this study is to characterise extraocular muscles in sloth bear from its habitat with behaviour. The right and left eyeball with intact extraocular muscles of six adult sloth bears were collected during post mortem examination of bears that had died due to natural causes and extraocular muscles were dissected and utilized for gross and histomorphological studies. Extraocular muscles of sloth bear comprised of dorsal (*M. rectus dorsalis*), ventral (*M. rectus ventralis*), lateral (*M. rectus lateralis*), medial rectus muscles (*m. rectus medialis*), the dorsal oblique (*M. obliquus dorsalis*) ventral oblique muscles (*M. obliquus ventralis*) and the retractor bulbi muscle (*M. retractor bulbi*) as like in domestic animals. Among extraocular muscles, m. dorsal oblique was the longest, m. lateral rectus was broadest and thinnest muscle and m. retractor bulbi was composed of four fascicles. The extraocular muscles were skeletal in nature, each muscle fascicle presented few homogenous slow twitch muscle fibers and more granular fast twitch muscle fibers. Nerve fiber bundles were more in number around the muscle fascicles in this study.

Keywords: extraocular muscles, sloth bear, gross, histology, histochemistry

Introduction
Bears are the youngest of the carnivore family, having arisen from dog-like ancestors during the Eocene Epoch, around 55 million to 38 million years ago. The sloth bear is originally thought to be a bear-like sloth due to its sloth like looks and behaviour. This bear is medium-sized and native only to southern Asia. These bears live in forests of India, Bhutan, Nepal and Bangladesh as well as in the island of Sri Lanka. The estimated number of bears still living in the wild is believed to be somewhere between 7000 and 10000. Sloth bears are classified as "Vulnerable" in the 1996 IUCN (International Union for Conservation of Nature and Natural Resources) Red List of Threatened Animals, and is listed on Appendix I of CITES (Convention on the International Trade in Endangered Species of Flora and Fauna). In India trade and export of sloth bears is illegal and the bears are completely protected under Schedule I of the Indian Wildlife Protection Act of 1972 as amended in 1986 (IUCN Red List, Version 2014.2). Bears have swaying head movement because of their habitat and behavior physiology. They have poor near vision (Joshi *et al*, 1995) [10]. They identify termites by using olfaction as a food resource. The present study was conducted on the gross, histological and histochemical studies on the extraocular muscles of sloth bear. Sloth bear are scheduled animals and live in forest, mountains and caves. They are rescued from the human beings who are using these animals as game animals. The objective of this study was to characterize extraocular muscles in sloth bear with behaviour.

Material and Methods
The eyeball with intact extraocular muscles of six adult sloth bears and six whole heads with intact eyes were collected during post mortem examination of bears that had died due to natural causes at the Wildlife Save Our Soul (WSOS), Bannergatta Bear Rescue facility Centre (BBRC), Bannerghatta National Park, Bengaluru and were utilized for gross and histomorphological studies.
The eyeballs were removed according to the method of Keller (1975) [12]. The eyelids were sutured together with continuous suture pattern from the medial to lateral canthus and 5 to 10

mililitre of 10 per cent Neutral buffered formalin infused into globe by using 18 gauge needle with syringe; the lids were then held and pulled out with an artery forceps and a scalpel knife was used to cut around the periorbital fat as traction was applied to the lids. This exposed the globe and further incision was made around the orbit, to reach the covering orbital fascia and extraocular muscles to reach the optic nerve and severing it. Further the eyeballs with intact orbital fascia and EOM were left in the 10 per cent NBF for 48 hours. The orbital fascia covering the eyeballs were carefully separated with the help of ophthalmic scissor and forceps from the eyeball to locate the EOMs. Carefully individual muscles were separated from the eyeball and length, width and thickness of each muscle were recorded using Vernier calipers, metal scale and non-absorbable thread (Joanna *et al*., 2003) [9]. After studying the morphological details of extraocular muscles, they were fixed in 10 % neutral buffered formalin for further histological and histochemical studies. The extraocular muscle tissues collected from sloth bear were processed by routine alcohol-xylene schedule and paraffin blocks were made (Luna, 1968) [18]. The sections were cut at 5-6 µm thickness and were stained by Haematoxylin and Eosin, Masson's trichrome method for collagen and muscle fibres (Luna, 1968) [18], Phosphotungstic Acid Haematoxylin (PTAH) method for skeletal muscles (Luna, 1968) [18], Gomori's sliver method for reticulum (Luna, 1968) [18], Bielschowsky's method for staining axis cylinders and dendrites (Luna, 1968) [18] and Periodic acid Schiff (PAS) technique for mucopolysaccharides (Luna, 1968) [18] study.

Results and Discussion

Carpenter (1977) [1], Konig and Liebich (2004) [14], Gellat *et al*. (2013) [4] and Evans and de Lahunta (2013) [3] reported in domestic animals that the muscles important to the function of the eye constitute three groups: intrinsic the extrinsic and the palpebral muscles. The intrinsic muscles regulate the pupillary diameter and the shape of the lens. The palpebral muscle group includes the muscles of the lid and of the head; these regulate the shape and position of the palpebral fissure. The extrinsic muscles are concerned with the movement of the eyeball; rotate the globe around three perpendicular axes. The complex movements require the fine coordination of all these muscles and they never act alone. In addition the eyeball can be retracted into the orbit along the optic axis by the retractor muscle of the eyeball.

In the present study extraocular extended from the sclera and to the wall of the orbit. Extraocular muscles in turn were covered by the thick and tough orbital/periorbital fascia. There were four recti muscles: the dorsal, ventral, medial, and lateral rectus (Figs.1&2). These recti muscles were inserted on the sclera posterior to the limbus in right and left eyeball at a distance of 4.2±0.05 mm and 4.3±0.05mm, respectively (Table-2). The dorsal oblique and ventral oblique muscles were inserted in an intermediate area between the insertions of the recti and retractor bulbi muscle. Muscle retractor bulbi was made up of four fascicles and all the four fascicles continued rostrally under recti muscles and formed a cone in appearance (Fig.3). These muscle fascicles surrounded the optic nerve and inserted posterior to recti muscles on the sclera. The space between recti muscles and retractor bulbi muscle was filled with intraperiorbital fat. Similar observations were made by Getty (1975) [5]; Konig and Liebich (2004) [14]; Gellat *et al*. (2013) [4] and Evans and de Lahunta (2013) [3] in domestic animals. The functional significance of these muscles is to move the eyeball in different directions. The integration between voluntary and involuntary control of eye occurs is a subject of continuing research. However it is known that vestibulo-ocular reflex plays an important role in the involuntary movement of the eye.

The average length of the dorsal rectus muscle on the right and left side was 5.10±0.19 cm and 5.13±0.16 cm, respectively (Fig.4 and Table.1). The average width of the dorsal rectus muscle on the right and left side was 1.11±0.16 cm and 1.11±0.11 cm, respectively. The average thickness of the dorsal rectus muscle on the right and left side was 0.25±0.02 cm and 0.22±0.01 cm. Kaseem *et al*. (1982) [11] assessed the dimensions of the dorsal rectus muscle in dog and it was 3.5 cm in length and 1 cm in thickness. Ridyard (2015) [21] observed 3.82 cm length of the superior rectus muscle in humans.

In the sloth bear the average length of the medial rectus muscle on the right and left side was 4.87±0.13 cm and 4.88±0.14 cm, respectively. The average width of the medial rectus muscle on the right and left side was 1.15±0.1 cm and 1.12±0.11 cm, respectively. The average thickness of the medial rectus muscle on the right and left side was 0.30±0.08 cm and 0.29±0.06 cm, respectively (Table-1). Kaseem *et al*. (1982) [11] mentioned the dimension of the medial rectus muscle in dog and recorded 3.2 cm in length and 0.9 cm in thickness. Whereas Ridyard (2015) [21] observed 3.85 cm length of the medial rectus muscle in humans.

The average length of the lateral rectus muscle on the right and left side was 4.80±0.10 cm and 4.83±0.09 cm, respectively. The average width of the lateral rectus muscle on the right and left side was 1.28±0.09 cm and 1.37±0.09 cm, respectively. The average thickness of the lateral rectus muscle on the right and left side was 0.14±0.09 cm and 0.16±0.06 cm, respectively (Table-1). Whereas Kaseem *et al*. (1982) [11] assessed the dimension of the lateral rectus muscle in dog and it was 3.9 cm in length and 0.97 cm in thickness. Ridyard (2015) [21] observed 3.84 cm length of the lateral rectus muscle in humans.

In the sloth bear the average length of the ventral rectus muscle on the right and left side was 4.78±0.11 cm and 4.73±0.12 cm, respectively. The average width of the ventral rectus muscle on the right and left side was 0.77±0.09 cm and 0.73±0.12 cm, respectively. The average thickness of the ventral rectus muscle on the right and left side was 0.20±0.02 cm and 0.22±0.07 cm, respectively (Table-1). Whereas in dog Kaseem *et al*. (1982) [11] reported the dimensions of the ventral rectus muscle as 3.4 cm in length and 0.9 cm in thickness. Whereas Ridyard (2015) [21] observed 3.72 cm length of the inferior rectus muscle in humans.

In the present study the average length of the dorsal oblique muscle on the right and left side was 6.24±0.12 cm and 6.26±0.09 cm, respectively. The average width of the dorsal oblique muscle on the right and left side was 0.29±0.06 cm and 0.30±0.03 cm, respectively (Table-1). The average thickness of the dorsal oblique muscle on the right and left side was 0.16±0.01 cm and 0.12±0.03 cm, respectively. Kaseem *et al*. (1982) [11] recorded the dimension of the dorsal oblique muscle in dog and it was measured 3.1 cm in length and 0.5 cm in thickness. Whereas Ridyard (2015) [21] observed 3.92 cm length of the superior oblique muscle in humans.

In the sloth bear the average length of the ventral oblique muscle on the right and left side was 5.15±0.13 cm and 5.17±0.09 cm, respectively. The average width of the ventral oblique muscle on the right and left side was 0.6±0.13 cm and 0.5±0.16 cm, respectively. The average thickness of the ventral oblique muscle on the right and left side was 0.16±0.05 cm and 0.18±0.08 cm, respectively (Table-1). Kaseem *et al*. (1982) [11] described the dimension of the ventral oblique muscle in dog and he recorded that the ventral oblique muscle was 3.6 cm in length and 0.6 cm thick. Whereas Ridyard (2015) [21] observed 2.25 cm length of the inferior oblique muscle in humans.

The average length of the retractor bulbi muscle on the right and left side was 4.47±0.19 cm and 4.48±0.16 cm, respectively. The average width of the retractor bulbi muscle on the right and left side was 0.47±0.09 cm and 0.45±0.10 cm, respectively. The average thickness of the retractor bulbi muscle on the right and left side was 0.25±0.04 cm and 0.23±0.07 cm, respectively (Table-1). Kaseem *et al*. (1982) [11] described the dimension of the retractor bulbi muscle in dog and he recorded that ventral oblique muscle was 3.4 cm in length and 0.4 cm thick.

According to Ridyard (2015) [21] muscle retractor bulbi is absent in humans. Ludinghausen *et al*. (1999) [17] observed the variations in the rectus muscles in humans which were thought to be the remnants of retractor bulbi and are responsible for preventing the protrusion of eyeball in most mammals.

The EOMs of sloth bear were oriented unidirectional. Each muscle fiber was separated by endomysium with large number of blood vessels and few nerve fibers (Fig. 5.6.7 &12). Between the fascicle nerve fiber bundles and blood vessels were also seen. In cross section muscle fibers appeared oval/round/irregular/polygonal with peripherally located nuclei with prominent nucleolus (Fig. 5.6&7). The muscle fiber showed uniform distribution of cytoplasm without stippled appearance hence they were considered to be fine fibers, which were few in number as compared to other muscle fibers, which were stippled without homogenous cytoplasm and hence they were considered to be coarse fibers. These fibers were more in number with irregular shape (Fig. 8&9). Based on these morphologoical features animal can be classified having slow twitch ocular muscle. Strong PAS positive reaction observed around the individual muscle fiber indicating more of mucopolysaccharide in the connective tissue (Fig. 9 and 10).

Three groups of muscle fibers were observed in sloth bear, large ones have homogenous cytoplasm while, medium and smaller ones have granular cytoplasm (Fig. 8&9). The muscle fibers having granular cytoplasm are considered to be fast twitch whereas nongranular muscle fibers (large) which are few in number can be considered as slow twitch. Around the muscle fibers reticular fiber network was seen, which was continuous with perimysium (Fig. 11). Based on the diameter muscle fibers can be divided into large, medium and small. The medium and small muscle fibers were irregular in shape and showed granular cytoplasm considered to be fast twitch (Fig. 8&9).

In conclusion it can be speculated that in sloth bear fast twitch muscles fibers are more as compared to slow twitch in the EOMs. Therefore the movement of the eyeball exhausted quickly and need rest. Kruger (1949) [15] identified fast and slow fibers based on the quantity of sarcoplasmic reticulum. Ogata (1960) [20] has classified the muscle fibers based on the activity of oxidative enzymes such as ATPase.

In the present study extraocular muscle fibers also showed the presence of PAS positive activity uniformly. In the present study all EOMs showed strong PAS positive but were negative for acidic and sulfated mucolpolysaccharide. Whereas Lakshmishree *et al*. (2008) [16] identified glycogen content as weak to moderate in EOMs of the buffalo.

Dempsey and Wislocki (1944) [2] suggested that the higher concentrations of glycogen in tissues accounts for low oxidative activity and provide energy through anaerobic pathway which is similar to the present finding showing higher concentration of glycogen in the EOMs.

The visual activity of the eyeball muscles are controlled by the brain in coordination with the retina as suggested by Jampel (2009) [8].

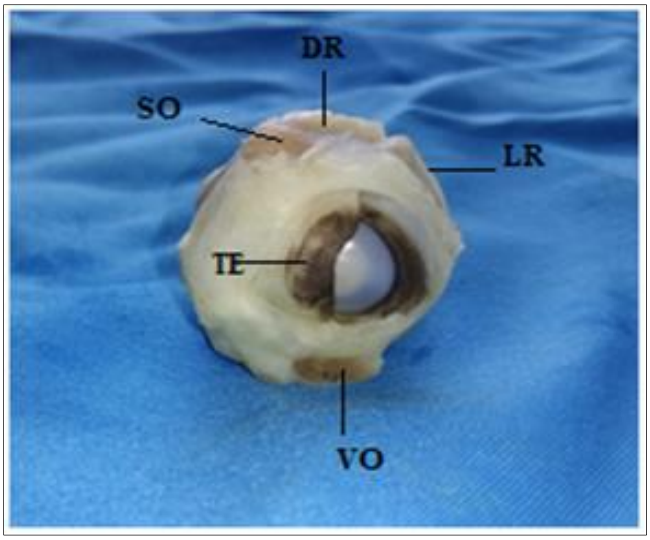

Fig 1: Photograph of sloth bear eyeball showing TE- third eyelid, DR- muscle dorsal rectus, SO-superior oblique, LR- lateral rectus, VO- ventral oblique.

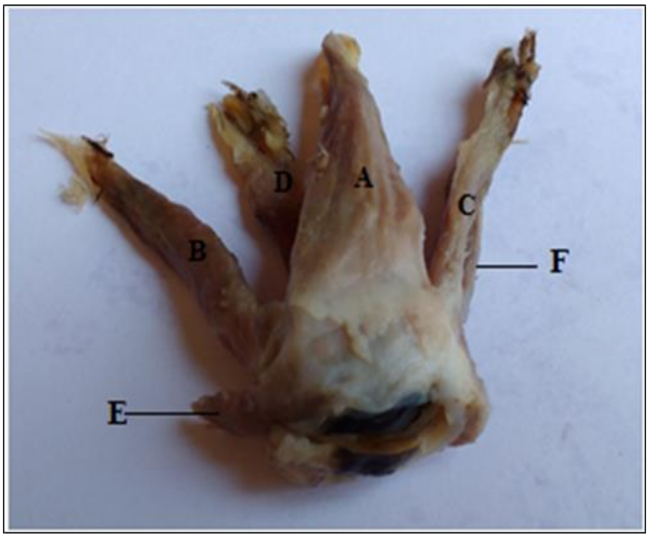

Fig 2: Photograph of lateral view of sloth bear eyeball showing EOM A- lateral rectus, B- dorsal rectus, C- ventral rectus, D- medial rectus, E- dorsal oblique and F- ventral oblique

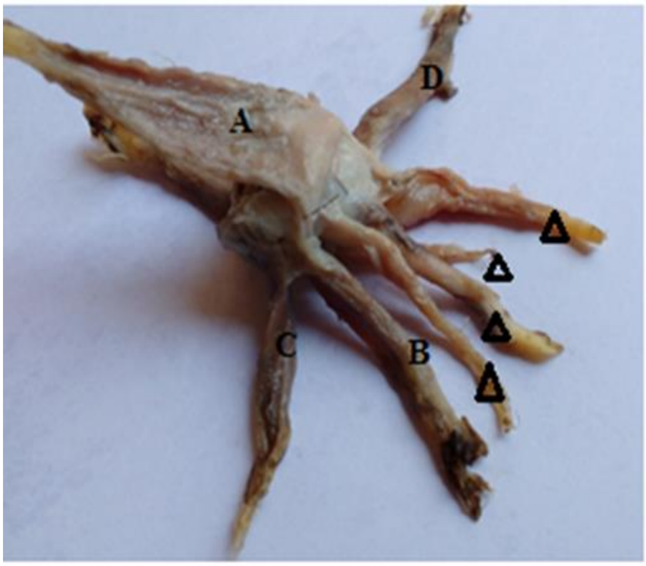

Fig 3: Photograph of sloth bear eyeball showing EOM A- lateral rectus, B- ventral rectus, C- ventral oblique, D- dorsal rectus and open arrow heads showing fascicles of retractor bulbi

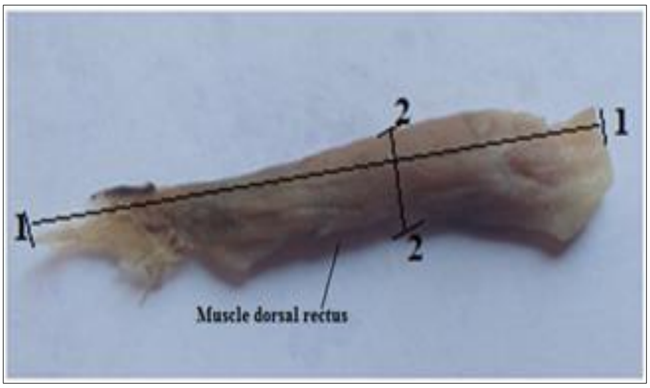

Fig 4: Photograph of muscle dorsal rectus of sloth bear showing 1-1- length of the muscle and 2-2- width of the muscle

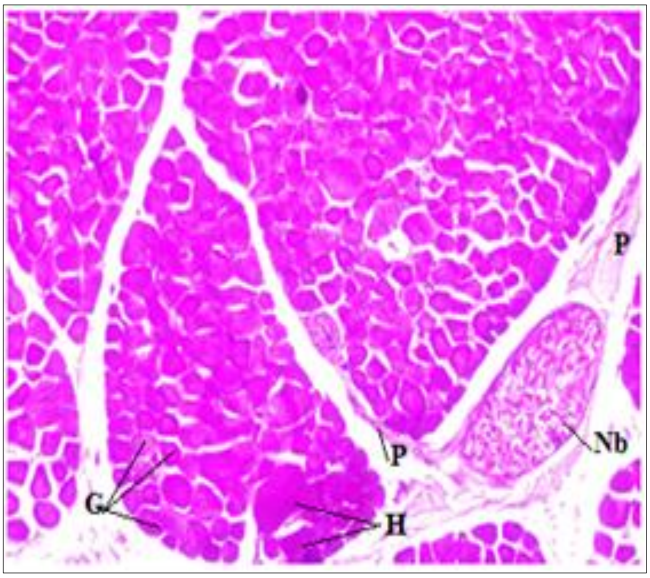

Fig 5: Photomicrograph of transverse section of muscle retractor bulbi showing G- muscle fiber with granular cytoplasm, H- muscle fiber with homogenous cytoplasm, P- perimysium and Nb- nerve bundle. (Haematoxylin and Eosin X100)

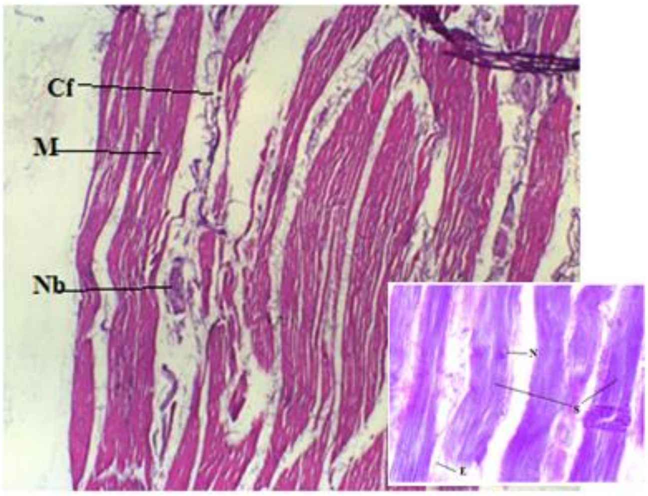

Fig 6: Photomicrograph showing horizotal section of muscle dorsal rectus M- muscle fibers orientation, Nb- nerve bundle and Cf- collagen fibers in perimysium (Haematoxylin and Eosin X100) and inset showing cross striations in lateral rectus muscle under PTAH stain X400

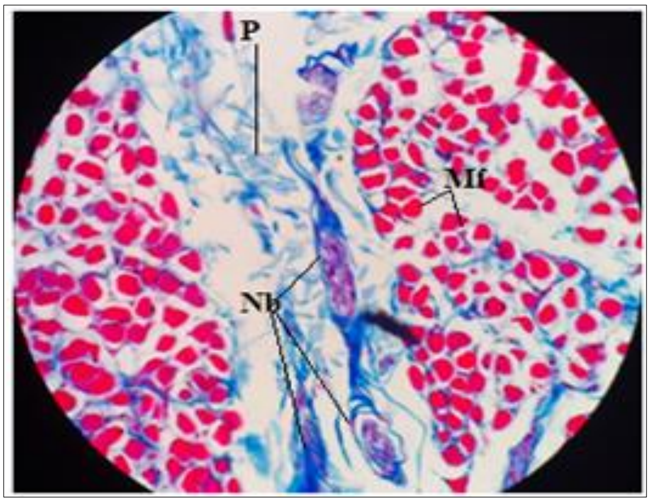

Fig 7: Photomicrograph of transverse section of muscle medial rectus showing P- Collagen fibers in perimysium, Mf- muscle fibers and Nb- nerve bundle (Masson's Trichrome X100)

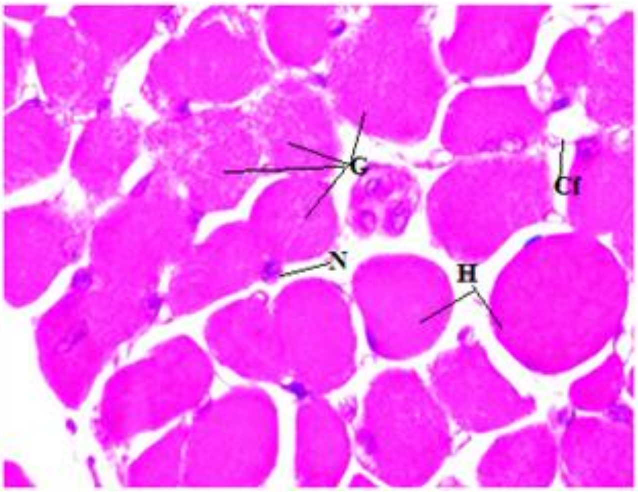

Fig 8: Photomicrograph of transverse section of muscle ventral rectus showing N- nucleus of muscle fiber, G- granular type of muscle fiber, H- homogenous type and Cf- collagen fiber (Haematoxylin and Eosin X400)

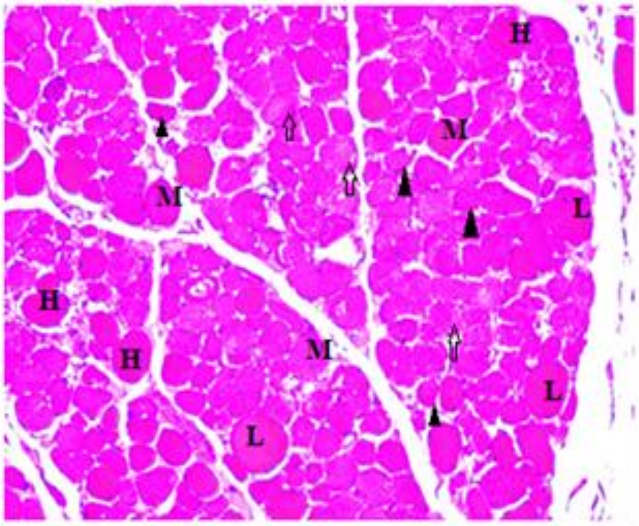

Fig 9: Photomicrograph of cross section of muscle ventral oblique showing L- large muscle fibers, M- medium and small muscle fibers (arrow heads) granular (open arrows) and H-nongranular type of muscle fibers (Haematoxylin and Eosin X100)

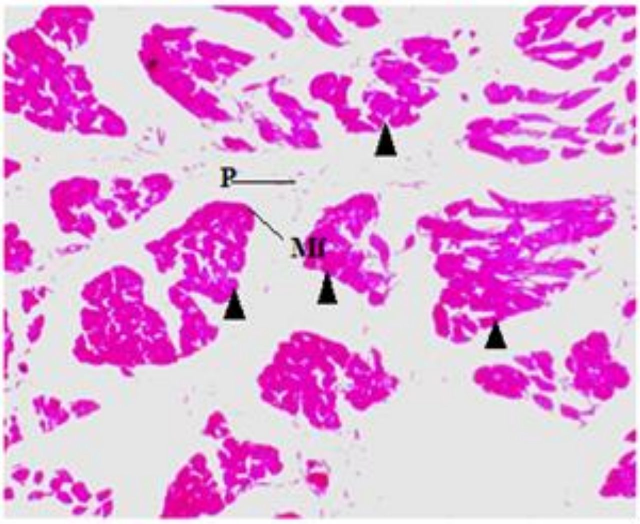

Fig 10: Photomicrograph of cross section of muscle ventral rectus showing positive reaction to PAS (arrow heads) P- perimysium and Mf- muscle fiber (Periodic acid Schiff X100)

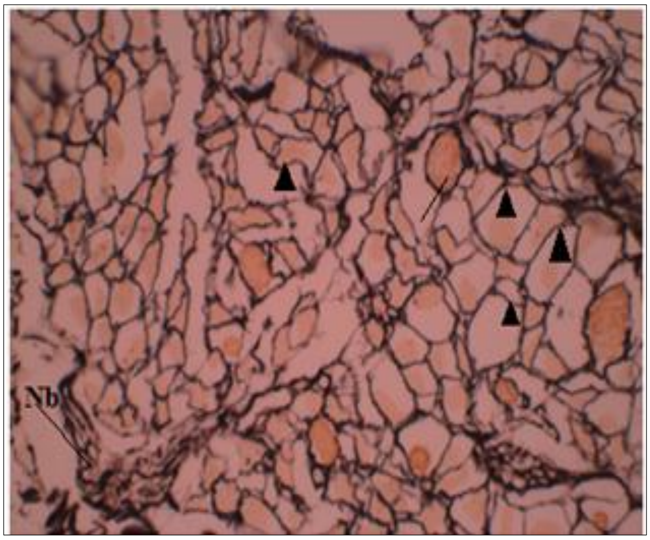

Fig 11: Photomicrograph of transverse section of muscle dorsal oblique showing reticular fibers (arrow heads) and Nb- nerve bundle (Gomori's X400)

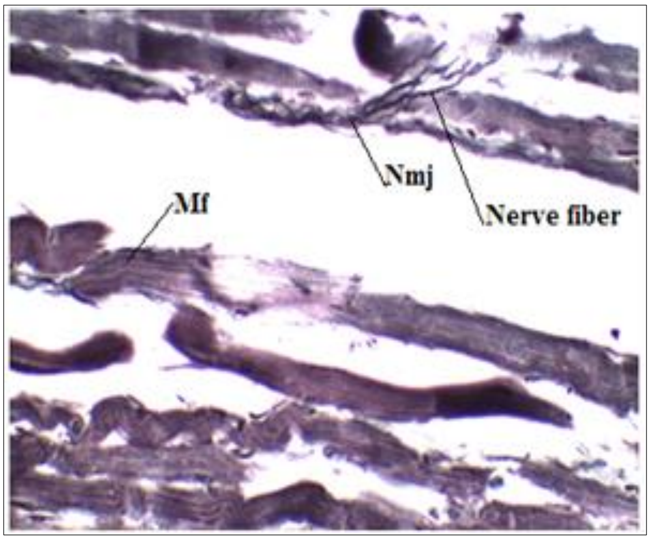

Fig 12: Photomicrograph of horizontal section of muscle medial rectus showing Nmj-neuromuscular junction and Mf- muscle fiber (Bielschowsky's X400)

Table 1: Morphometry of extraocular muscles in both eyes

Sl. No	Name of muscle	**Right** (cm)			**Left**(cm)		
		Length	**Width**	**Thickness**	**Length**	**Width**	**Thickness**
1	Dorsal rectus	5.10±0.19	1.11±0.16	0.25±0.022	5.13±0.16	1.11±0.11	0.22±0.01
2	Medial rectus	4.87±0.13	1.15±0.1	0.30±0.08	4.88±0.14	1.12±0.11	0.29±0.06
3	Lateral rectus	4.80±0.1	1.28±0.09	0.14±0.09	4.83±0.09	1.37±0.09	0.16±0.06
4	Ventral rectus	4.78±0.11	0.77±0.09	0.2±0.02	4.73±0.12	0.73±0.12	0.22±0.07
5	Dorsal oblique	6.24±0.12	0.29±0.06	0.16±0.01	6.26±0.09	0.3±0.03	0.12±0.03
6	Ventral oblique	5.15±0.13	0.6±0.13	0.16±0.05	5.17±0.09	0.5±0.16	0.18±0.08
7	Retractor bulbi	4.47±0.19	0.47±0.09	0.25±0.04	4.48±0.16	0.45±0.10	0.23±0.07

Table 2: Distance between the limbus to point of insertion of recti muscles of Sloth Bear

Animal number	Right eyeball (mm)	Left eyeball(mm)	P value
1	4.4	4.3	0.20
2	4.1	4.2	
3	4.2	4.2	
4	4.1	4.4	
5	4.3	4.2	
6	4.1	4.5	
Mean±SE	4.2±0.05	4.3±0.05	

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