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RESEARCH ARTICLE

# Gross and microanatomical studies on the moderator bands (septomarginal trabecula) in the heart of mature Dromedary camel (*Camelus dromedarius*)

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## ABSTRACT

**The current work was carried out to investigate the gross and microanatomical features of moderator bands (septomarginal trabecula) in camel heart. Ten hearts were collected from healthy mature dromedary camels. Anatomically, the moderator bands were present in both right and left ventricles. In right ventricle, the walls had one muscular moderator band which was extended from the interventricular septum to the opposite ventricular wall especially to the papillary muscle. In left ventricle, there were two bands; one extended from the interventricular septum to the papillary muscles, and the other one was present in various places especially in the apex running as a thin thread-like band across the left ventricular wall. Histological examination revealed that the moderator band consisted of two major layers; the central (core) myocardium and the peripheral endocardium, acting as band capsule. The myocardium had two bundles; the contractile cardiac muscle bundles and the Purkinje fiber bundles. The endocardium consisted of three layers; the endothelial layer of simple squamous epithelium, the subendothelial layer of loose connective tissue and the subendocardial layer, connecting the endocardium with the myocardium.**

## Keywords:

Dromedary camel, heart, moderator band, Purkinje fiber, septomarginal trabecula, ventricular trabecula

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## INTRODUCTION

Moderator bands (septomarginal trabecula) are fibromuscular structures crossing the ventricular cavities without being attached to the cusps (Cocchieri and Bardelli, 1992). The term 'moderator band' is in accordance with modern anatomical nomenclature (Nomina Anatomica Veterinaria, 2005). Anatomically, Crick et al. (1998) reported the presence/location of moderator bands in both ventricles of the pig's heart. Iakimov (2009) assumed that the differences between the right and left ventricular trabecular patterns were conditioned by the changes of intracardiac blood flow during the prenatal period.

Rocha et al. (2010) have shown that the muscle bundle in the septomarginal trabecula of pig heart is generally a resistant crest that goes from the lower part of the septum to the lower part of the anterior papillary muscle. Sathyamoorthy and Ramesh (2008) claimed the moderator band for being composed of an outer thick endocardial covering, Purkinje fibers, ordinary myocardial fibers, blood vessels and nerves. The endocardium was thick and consisted of an endothelial covering and an underlying connective tissue of collagen, elastic and a few reticular fibers. The Purkinje fibers were large, irregular cells with centrally placed nuclei. It was present either in the myocardium or in the subendocardium.

The histological examinations have shown moderator bands to be consisted primarily of cardiac muscle fibers with slight vascularization. On cross-section of the moderator band, clumps of conductive cells were observed in the peripheral and central parts of the band

around the blood vessels. In the large moderator band, conductive cells were found around the vessels and long Purkinje fibers were also found at the periphery of the band and in the central part, next to the vessel (Gulyaeva and Roshchevskaya, 2012). Lotkowski et al. (1997) and Loukas et al. (2008) assumed that Purkinje fibers acted as an extension of the His bundle branches inside the moderator bands of calf and human ventricles, forming an intracavitary branching of the cardiac conduction system. Gulyaeva and Roshchevskaya (2012) identified the presence of large amount of muscle fibers in all the bands and also showed clumps of Purkinje cells located both at the periphery and in the central part around the vessels. Moreover, standard anatomical texts, in description of the ventricles, it was described the band as a portion of the ventricular conduction system involving the right atrioventricular bundle, as conduction tissue fibers and Purkinje fibers moved toward the apex of the ventricle before entering the anterior papillary muscle (Standring, 2005). Okamoto et al. (1981) clarified that the moderator bands primarily provided quick transmission of electric impulses to the parietal wall and prevent excessive dilatation of the ventricles during diastole. Kervancioglu et al. (2003) and Deniz et al. (2004) described that the moderator bands of both sheep and goat were consisted of connective tissue and conduction system fibers. Only 20% of sheep moderator bands contained muscle fibers and blood vessels, and no muscle fibers were found in the bands of goat hearts. Furthermore, Gulyaeva and Roshchevskaya (2012) described that the muscle fibers form the largest part of the moderator band section in the pig's right ventricle and conductive cells were few in number, so the main function of the band is not to conduct electric impulses but rather to prevent excessive ventricular dilatation during diastole through muscle fibers tension. However, few reports are available that describe the moderator bands of camel. Therefore, the research work was focused on anatomical and histological aspects of the moderator bands of camel heart ventricles that would enable in establishing the cardiac myocytes and the Purkinje fibers as regular constituents of camel heart.

## MATERIALS AND METHODS

Hearts of ten apparently healthy mature camels were collected from Zagazig slaughter house in Sharkia province, Egypt for anatomical and histological studies. For light microscopy, the moderator bands were immediately fixed in 10% buffered neutral formalin and Bouin's fluid. The fixed specimens were processed using the usual histological techniques. Briefly, specimens were dehydrated in ascending grades of ethanol series, cleared in benzene and embedded in paraffin. Tissue sections (5-7 µm in thickness) were prepared and mounted on glass slides. These sections were dewaxed in xylene, hydrated in descending grades of ethanol series and stained with Harris's hematoxylin and eosin (H&E) stain for histological studies (Bancroft and Gamble, 2001).

Periodic acid schiff technique (PAS) was used for the detection of neutral mucopolysaccharides (Bancroft and Gamble, 2001). The microphotography was done by using a digital Dsc-W 130 super steady cyber shot camera connected to an Olympus BX 21 light microscope.

## RESULTS

Anatomically, the moderator band was a single or branched fibromuscular strand that extended from the interventricular septum to the opposite ventricular free wall at the papillary muscle, passing through the ventricular cavity and appeared as fleshy and muscular in its consistency (Figure 1A, B, C). The moderator bands were found in both right and left ventricles of the camel heart. In the right ventricle, the walls had one muscular moderator band; ventricular septum which extended from the interventricular septum to the opposite ventricular wall especially at the papillary muscle (Figure 1B, C). In the left ventricle, moderator bands were two; one was extended from the interventricular septum to papillary muscles (the larger one) while the other one was present in various places especially in the apex running as a thin thread-like band. These bands in the left ventricle run inside the ventricular wall. On the other hand, in the right ventricle, the moderator band emarginated and evaginated from the septum to the opposite wall crossing and passing through the ventricular cavity, not inside the ventricular wall (Figure 1D, E).

Histologically, the moderator band was observed consisting of two major layers; the central (core) myocardium and the peripheral endocardium, acting as moderator band capsule. The moderator band endocardium was considered the outermost layer; a capsule, completely surrounding and covering the band from all directions. The endocardium was consisted of three layers including endothelial layer, subendothelial and subendocardial layers. From outside directing to the inside, the moderator band was completely covered externally by a single layer that was reflected from the ventricular endothelium

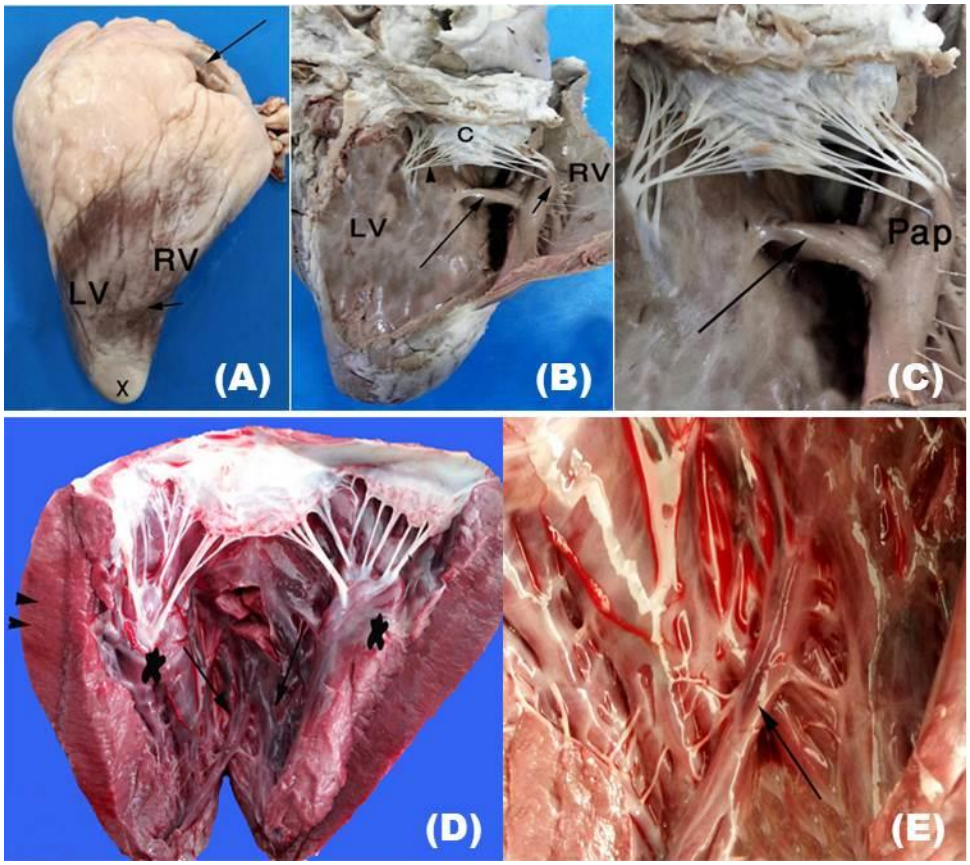


**Figure 1. Gross anatomical features of moderator bands in the mature dromedary camel heart ventricles.** **(A)** Mature camel heart showing the right atrium (long arrow), the right ventricle (RV), the right ventricular end (short arrow), left ventricle (LV) and the heart apex (X), **(B)** Opened right ventricle (RV), papillary muscle (short arrow), chordae tendinae (arrow head), cusps of the valve (C), the moderator band (long arrow) and the left ventricle (LV), **(C)** High magnification of 1(B) showing the extension of the moderator band from the inter ventricular septum to the papillary muscle passing through the right ventricular cavity (long arrow), the papillary muscle (Pap), **(D)** Mature camel left ventricle showing the opened left ventricle with thick wall (arrow head), moderator bands (arrow), papillary muscles (star), **(E)** High magnification of 1(D) showing the extension of the moderator band inside the left ventricular wall in the form of thin cords (arrow).

(Figure 2A, C, D). The subendothelial layer was the second layer of the endocardium that supported the endothelium and consisted of narrow zone of loose connective tissue (Figure 2A, C, D). It showed strongly PAS positive reaction (Figure 2B). Moreover, no clear cut border could be seen separating the endothelium and subendothelium (Figure 2A, B, C, D).

The subendocardial layer was the innermost layer of the endocardium that connected the endocardium with the myocardium. It was composed of loose connective tissue with two types of modified cardiac muscle cells. These modified cardiac muscle cells were considered the developmental stages of Purkinje fibers with different sizes and shapes staring from the perinuclear clear zone cells that in cross section were observed round or ovoid with perinuclear clear zone and had few myofibrils in the cytoplasm with single, central and large nucleus (Figure 2A, C). These cells were gradually increasing in size, with larger perinuclear clear zone, and fewer myofibrils, arranged only at the peripheral parts of the cells, making the cell cytoplasm paler and forming small Purkinje-like cells. The latter were observed continuously increasing in its size and becoming larger and larger till reaching to the giant Purkinje fibers that had same characters of the ventricular Purkinje fibers. The moderator band Purkinje fibers were observed round, larger in size than the perinuclear clear zone cells and also larger than the cardiac myocytes. Its cytoplasm was pale as it contained very few myofibrils enriched with glycogen. It had a single, central, large nucleus and sometimes binucleated with prominent nucleoli (Figure 2A, C, D). Moreover, these cells were highly reactive to PAS showing the presence of large amount of glycogen in its cytoplasm (Figure 2A).

The central layer was considered the normal myocardium layer like that in the ventricular wall where it consisted of contractile cardiac muscle fibers, arranged longitudinally from the interventricular septal myocardium to the moderator band and then to

the myocardium of the ventricular wall, forming groups of longitudinal cardiac muscle bundles (Figure 2E). The longitudinal bundles of cardiac muscle cells were laterally separated from each other by a considerable amount of loose connective tissue. Moreover, it was rich with a dense capillary network, lymph vessels and autonomic nerve fibers (Figure 2J, K). This intercellular connective tissue reacted positively with PAS (Figure 2B). The intercellular connective tissue was few where the myocardiocytes in the moderator band were very large in number and size, closed to each other and appeared overcrowded. Moreover, most of these cells were elongated, branched and connected with each other, forming some sort of network-like structure (Figure 2B, E, F, H).

The cardiac muscle cells in longitudinal sections were long, striated, branched and anastomosed, forming network of the myocardial fibers. Furthermore, most of the cardiac muscle cells possessed only a single, relatively large, oval, ovoid pale-staining with more euchromatic and centrally placed nucleus; however, some binucleated cells were occasionally observed, occupying a central position in the muscle cells. The nuclear chromatin was dispersed and in most cases tended to be condensed peripherally (Figure 2E, F, G). Meanwhile, in the cross sections, the cardiac muscle fibers appeared with irregular polygonal cells of various sizes with a large, round, pale-staining, euchromatic, centrally placed, single nucleus (Figure 2I). Moreover, the myocardiocytes also showed a PAS positive reaction (Figure 2G). The cardiac muscle sarcoplasm was found eosinophilic and full of parallel contractile myofibrils that were consisted of myofilaments. They exhibited a very stronger cross-striated banding pattern (Figure 2F).

Beside the longitudinal cardiac muscle bundles, the Purkinje fibers were present and organized into bundles in between and surrounding the cardiac muscle bundles, filling the core of the moderator band. These Purkinje cells were observed round, larger in size than the working cardiac myocytes, showing variety in number and size. Its cytoplasm was pale as it contained very few myofibrils than normal myocardiocytes and rich in glycogen. It had single, central, large nucleus and sometimes binucleated with prominent nucleoli (Figure 2O, P). Moreover, these cells showed strong PAS positive reaction and some PAS positive granules were also identified, especially within the central pale cytoplasmic areas (Figure 2N, O).

The Purkinje cells were surrounded by a connective tissue sheath (Figure 2O). Moreover, the intercellular connective tissue showed positive reaction with PAS (Figure 2P). In some sections, there were some distributions of fat cells, merged together forming bundles of fat cells. The latter, was housed inside the loose connective tissue, surrounding the Purkinje fibers (Figure 2L, M). Some individual fat cells were also observed within the myocardium in between the cardiac myocytes (Figure 2L).

## DISCUSSION

The current work revealed that the moderator band is a single or branched fibromuscular strand. These findings are in line with Cocchieri and Bardelli (1992) who described that the moderator bands are fibromuscular structures crossing the ventricular cavities without being attached to the cusps. The moderator bands was found in both right and left ventricles in the camel heart. This finding was in agreement with Crick et al. (1998) in pigs. In the right ventricle, the walls had one muscular moderator band which extended from the interventricular septum to the opposite ventricular wall especially at the papillary muscle. This was in coincidence with Lorenz and Hunigen (1989), Lorenz (1990), Lorenz and Guski (1990), Gulyaeva and Roshchevskaya (2012) in pigs and Parto et al. (2010) in ostrich.

The function of the band was to prevent over distention and dilatation of the right ventricle during contraction. These investigations are the same as the findings of Okamoto et al. (1981) in human, Parto et al. (2010) in ostrich and Gulyaeva and Roshchevskaya (2012) in pigs. The moderator bands in the left ventricle were two; one extended from the inter ventricular septum to papillary muscles while, the other one present in various places especially in the apex but these bands run inside the left ventricular wall. These investigations are in a similarity with Nickel et al. (1981) in domestic animals, Parto et al. (2010) in ostrich and Gulyaeva and Roshchevskaya (2012) in pigs. The moderator band was consisted of two major layers; the central myocardium and the peripheral endocardium, acting as the moderator band capsule. This finding was in close agreement with Parto et al. (2010) who reported that the moderator bands are consist of longitudinal muscle fibers in central with Purkinje cells in peripheral and are covered by endocardium in ostrich.

The endocardium consisted of three layers; the endothelial layer, the subendothelial layer and the subendocardial layer. The moderator band was completely covered externally by a single layer of simple squamous epithelium. This endothelium was

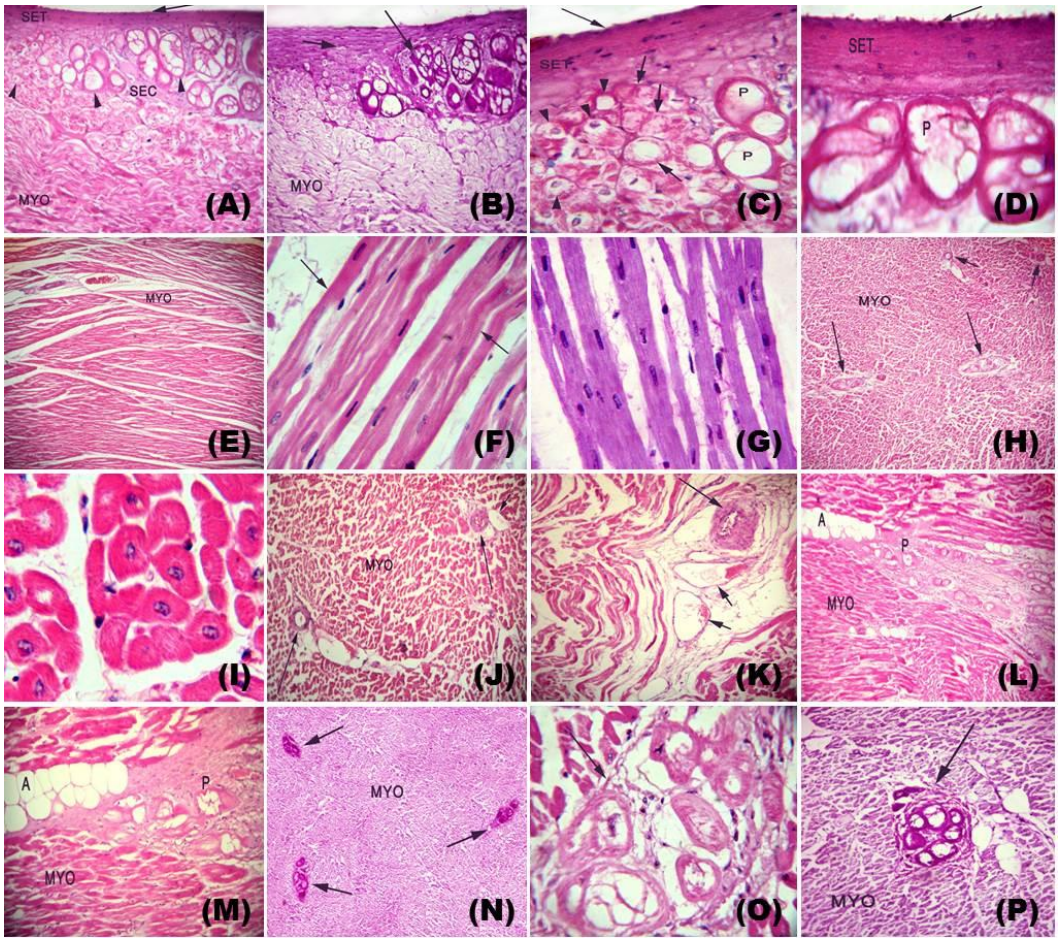


**Figure 2. Micro-anatomical features of the moderator bands in the mature dromedary camel heart ventricles. (A)** Endothelium (arrow), the subendothelium (SET), the subendocardium (SEC), Purkinje fibers (arrow head) and myocardium (MYO). Stain: H&E (x100), **(B)** Highly PAS positive reaction of the subendothelium (short arrow) and subendocardium (long arrow) and low PAS reaction of the myocardium (MYO) Stain: PAS (x100), **(C)** Moderator band showing the endothelium (long arrow), the subendothelium (SET), the developmental stage of the Purkinje fibers (arrow head, short arrow, P), Purkinje fibers (P). Stain: H&E (x400), **(D)** Endothelium (arrow), the subendothelium (SET), the Purkinje fibers (P). Stain: H&E (x1000), **(E)** Longitudinal section of the moderator band showing the one direction of the cardiac muscle within the myocardium (MYO). Stain: H& E (x40), **(F)** Longitudinal section of the cardiac muscle with the striation (arrow). Stain: H& E (x400), **(G)** Moderately PAS positive reaction of the cardiac muscle Stain: PAS (x400), **(H)** myocardium (MYO), BVs (short arrow) and Purkinje bundles (long arrow). Stain: H&E (x40), **(I)** C.S. of the cardiac muscle within the myocardium. Stain: H& E (x400), **(J)** Myocardium (MYO), BVs (arrow). Stain: H&E (x40), **(K)** BVs (long arrow), lymph vessels (short arrow). Stain: H&E (x100), **(L)** myocardium (MYO), Purkinje fibers (p) and fat cells (A). Stain: H&E (x40), **(M)** High magnification of Figure 2(N) showing myocardium (MYO), Purkinje fibers (p) and fat cells (A). Stain: H&E (x100), **(N)** Moderate PAS reaction of the cardiac muscle within the myocardium (MYO) and highly PAS reaction of the Purkinje bundles (arrow). Stain: PAS (x40), **(O)** Purkinje bundle within the myocardium. Stain: H&E (x400), **(P)** Myocardium (MYO), Purkinje bundle (arrow). Stain: PAS (x100).

considered the outer most layer of the endocardium that was supported by a subendothelial loose connective tissue. This finding was in close agreement with Parto et al. (2010) in ostrich and Sathyamoorthy and Ramesh (2008) in horses clarifying that the endocardium is thick and consisted of an outer endothelial layer situated over a fibro-elastic thick layer of subendothelium that is composed of collagen, elastic and a few reticular fibers.

The subendocardial layer was the innermost layer of the endocardium and composed of loose connective tissue, having many blood vessels, lymph vessels, nerves. It had two types of modified cardiac muscle cells; the perinuclear clear zone cells and Purkinje fibers. This finding was in line with Parto et al. (2010) in ostrich and Sathyamoorthy and Ramesh (2008) in horses that clarified that the subendocardial connective tissue layer is loose and consisted of blood vessels and Purkinje fibers at some places. Moreover, this is in agreement with the findings of Dellmann and Eurell (1998) in some other animals. The moderator band Purkinje fibers were round in shape, larger in size than the perinuclear clear zone cells and cardiac myocytes.

Its cytoplasm was pale as it contained very few myofibrils, enriched with glycogen. It had single, central and large nucleus. This finding was in close agreement with Parto et al. (2010) in ostrich and Sathyamoorthy and Ramesh (2008) in horses who clarified that the Purkinje fibers were dispersed around the periphery with a vacuolated appearance in the center of the cell. The nuclei were round to oval and centrally placed, surrounded by a vacuolated spaces that was occupied by the glycogen.

The central layer was consisted of contractile cardiac muscle fibers that were arranged longitudinally from the interventricular septal myocardium to the moderator band and then to the myocardium of the ventricular wall and also run inside the papillary muscles, forming groups of longitudinal cardiac muscle bundles. This result was indicating that the cardiac muscle fibers in the interventricular septum, the moderator band, the ventricular wall and the papillary muscle were the same. These results agreed with the findings of Nickel et al. (1981) in domestic animals and Parto et al. (2010) in ostrich who stated that in the right and left ventricles, the walls have a muscular moderator band, extending from the interventricular septum to the opposite ventricular wall especially at the papillary muscle. The function of this structure was to prevent over distention and dilatation of the right ventricle during contraction and to allow the Purkinje fibers to extend from the atrioventricular bundle branch to the papillary muscles and myocardium of right and left ventricular parietal wall. Moreover, this investigation was in line with those of Abdulla et al. (1990) and Deniz et al. (2004) in human who demonstrated that moderator band extended between interventricular septum and ventricular free wall in man.

The longitudinal bundles of cardiac muscle cells were laterally separated from each other by a considerable amount of loose connective tissue. Moreover, it was enriched with a dense capillary network, lymph vessels, and autonomic nerve fibers. This was consistent with the findings of Mescher (2010) and Ghallab (2000) who stated that the loose connective tissue was arranged around and between muscle fibers and bundles to transmit the pull of the muscle to its attachment. It carried blood vessels, lymphatics and nerves to muscle cells. A delicate highly vascularized loose connective tissue; endomysium was appearing as slit-like regions between the muscle fibers. The intercellular connective tissue was reacted positively with PAS. The intercellular connective tissue was few where the myocardiocytes in the moderator band were very large in number and size and also much closed to each other and appeared overcrowded. Most of these cells were elongated, branched and connected with each other, forming some sort of network-like structure. This finding was in close agreement with Marei et al. (1994) in camel, Dellmann and Eurell (1998) in bovines, Cormack (2001) in human, Eurell (2004), Eurell and Frappier (2006), Samuelson (2007) in bovines, Junqueira et al (2007), and Gartner and Hiatt (2007) in human.

The cardiac muscle cells in longitudinal sections were appeared long, striated, branched and anastomosed, forming network and joined end to end and side to side at intercalated disks, forming the myocardial fibers. Furthermore, most of the cardiac muscle cells possessed only a single, relatively large, oval, ovoid pale-staining, more euchromatic and centrally placed nucleus, however, some binucleated cells were also occasionally observed, occupying a central position in the muscle cell and some of them showed prominent nucleoli. The nuclear chromatin was dispersed and in most cases tended to be condensed peripherally. This result was similar to the findings of Youssef et al. (1988) in albino rats and Marei et al. (1994) in camel. Meanwhile, in the cross sections, the cardiac muscle fibers appeared irregular polygonal cells of various sizes with a large, round, pale-staining, euchromatic, centrally placed, single nucleus and also sometimes, binucleated cells.

The cardiac muscle sarcoplasm was eosinophilic and full of parallel contractile myofibrils that were consisted of myofilaments. They exhibited a very stronger cross-striated banding pattern than in the atrium; where, the sarcoplasm showed its characteristic striations of alternating dark and light bands. These results were similar to the findings reported by Marei et al. (1994) in camel. Beside the longitudinal cardiac muscle bundles, the Purkinje fibers were present and organized into bundles in between and surrounding the cardiac muscle bundles, filling the core of the moderator band. This result was parallel with the findings of Sathyamoorthy and Ramesh (2008) in horses and Parto et al. (2010) in ostrich, who assumed that beside the longitudinal cardiac muscle bundles, bundles of Purkinje fibers were present in between and surrounding the cardiac muscle bundles, filling the core of the moderator band.

These Purkinje cells were round in shape, larger in size than cardiac myocytes, showing variety in number and size. Its cytoplasm was pale as it contained very fewer myofibrils than normal myocardiocytes. It had single,

central, large nucleus and sometimes binucleated with prominent nucleoli. The nuclear chromatin was dispersed and in most cases tended to be condensed peripherally. However, some non-nucleated cells were also observed. Moreover, these cells showed strong PAS positive reaction where some PAS positive granules were identified. This finding was in close agreement with Marei et al. (1994) in camel. There was cell-to-cell communication between Purkinje fibers within the bundle. Moreover, these Purkinje cells were surrounded by a connective tissue sheath. This finding wasconsistent with that of Parto et al. (2010) in ostrich. Moreover, the intercellular connective tissue also showed positive reaction to PAS and this was in agreement with the findings of Marei et al. (1994) in camel.

## CONCLUSIONS

Based upon findings, it is concluded that the moderator bands were present in both right and left ventricles of the dromedary camel heart. Histologically, the moderator band was consisting of two major layers; the central myocardium and the peripheral endocardium which acted as moderator band capsule. Moreover, bundles of Purkinje fibers were present in between the contractile cardiac muscle bundles within the moderator band myocardial layer.

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