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HISTOMORPHOMETRIC EVALUATION OF DROMEDARIAN (*Camelus dromedarius*) HEART

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ABSTRACT

The primary objective of present study was comprehensive exploration of age related cardiac anatomy of camel which might be helpful for appropriate understanding of CVDs. Seven hearts of each, young (1-2 years) and adult (>5 years), group were collected and dissected for macroscopic and microscopic evaluation. Tissue sections were prepared by paraffin tissue technique and morphometry was done using Image J ®. Means of all parameters were compared by student T-Test. In macroscopic evaluation relative weight of heart was found significantly ($P<0.05$) higher in young dromedaries while absolute weight, circumference, thickness of left ventricle wall, width of moderator band and thickness of aortic wall along with lumen were found significantly ($P<0.05$) higher in adult dromedaries. Microscopic parameters of heart like thickness of endocardium, sub-endocardium and the purkinje fibres diameter were found significantly ($P<0.01$) thicker in adults than young dromedaries; however, it was otherwise for sub-endothelium. Parenchymal percentage in myocardium was found significantly ($P<0.01$) lower in young than adult dromedaries, however, the stromal content followed the inverse pattern. These cardiac anatomical results can be implicated for legitimate understanding of CVDs.

Key words: Camels, Heart, Histomorphometry

Cardiovascular diseases (CVDs) are the class of complications in which normal anatomy and physiology of heart along with arteries and veins are badly affected. Cardiac arrest is most common complication of faulty coronary circulation. Constraints in the coronary circulation lead to local ischaemia, acidosis (intra and extracellular), anaerobic glucolysis along with faulty membrane permeability of cardiac muscles (Custodis *et al*, 2013; Lelovas *et al*, 2014). Macroscopic and microscopic data in quantitative form on cardiovascular system organs i.e., heart, arteries, veins and blood is considered critical for the legitimate understanding of etiology and pathogenesis of heart related diseases (Qureshi *et al*, 2017).

The demand for a biomedical model that precisely approximates the human cardiovascular anatomy and physiology is indispensable. In recent era of biomedical research large animals are being focused to understand the CVDs like dogs and porcine (Lelovas *et al*, 2014).

Dromedaries are famous for their unique physiological adaptation in extreme conditions in which other animals cannot withstand. The normal physiological circulatory vitals of camelids are blood pressure of 76-115 mmHg with cardiac rate 50 beats/min. Camel contains more blood volume (93 ml/kg) than those of other livestock animals. About 15000 ml blood can be collect from 400 kg heavy camel after bleeding (Ouajd & Kamel, 2009).

The primary objective of present study was comprehensive exploration of age related cardiac anatomy of camel which might be helpful for appropriate understanding of CVDs in camels and other species.

Materials and Methods

This study included 14 clinically healthy dromedaries of either sex of two age groups (n=7) *i.e.*, young (1 to 2 years) and adult (more than 5 years). Live weight of these animals was calculated by using formula as follows: Live weight (kg) = S(m) x T(m) x A(m) x 52 ± 25kg given by Abebe *et al* (2002).

Where S: shoulder height, T: thoracic girth and A: abdominal girth m: meter.

Before slaughtering, dentition was used to estimate the age of the animals according to the dentition formula as described by Rabagliati (1924). Different physiological parameters like rectal temperature, respiration rate and pulse rate were determined in each animal before slaughter to ascertain health status of animals (Table 1). The

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heart specimens were collected from local abattoir immediately after slaughtering from October to December, 2016. Following the collection, these were transferred to the gross anatomy laboratory of Anatomy Department at University of Agriculture, Faisalabad. The weight of the heart was determined using digital weighing balance. Relative weight was worked out by using total body weight and heart weight. Different cardiac morphometric parameters like shape, colour of coronary fat, length (from base to apex) and circumference were measured by using measuring tape. The cardiac samples were dissected from right and left sides by using surgical scalpel for atrial and ventricular dimensions. Thickness of wall of atria and ventricles of both sides were measured by using Vernier's caliper. Along with these, thickness of moderator band, thickness of aortic wall and aortic lumen were also recorded. For microscopic evaluation, 1-2 cm^3 large tissue samples were dissected from left and right atria and ventricles immediately after slaughtering and fixed in buffered formalin after washing with normal saline. Tissues were cut into thin slices and processed by paraffin preparation technique. Sections were cut at 5 µm thickness and subjected to Hematoxylin and Eosin (H&E) staining procedure as described by Bancroft *et al* (2013). Prepared slides were subjected to Image J® analysis (version 1.46) software for measurement of histological parameters like thickness (µm) of endocardium, sub-endothelium and sub-endocardium, diameter (µm) of purkinje fibres, percentage stromal and parenchymal content of myocardium.

Statistical analysis:

Means, standard error of means (SEM) and ranges for each parameter were computed using Microsoft Excel®. Group means of young and adult animals were compared by Student's T-test done with the statistical software Minitab®. Group means were compared at 5 per cent level of confidence.

Results and Discussion

Macroscopic parameters:

The heart of dromedaries was reddish brown in colour, conical shaped with white coloured coronary fat regardless of their age (Fig 1). The mean ± SEM values of all macroscopic parameters are given in Table 2. Statistical findings showed that the mean values of absolute weight of heart were found significantly ($P<0.05$) higher in adult dromedaries as compared to young dromedaries while this situation was found reverse in case of relative weight of heart. The length of heart was found non-significantly ($P>0.05$) different in both groups and adult dromedaries had significantly ($P<0.05$) larger circumference as compared with young dromedaries. The left ventricle wall thickness was found much more than that of right's within the same heart (Fig 1). The thickness of right ventricle wall was found statistically similar ($P>0.05$) in both groups, however, left ventricle wall thickness was significantly ($P<0.05$) thicker in adult camelids. A similar trend was observed in case of moderator band width while its thickness remained non-significant ($P>0.05$) in both groups. The descending aortic lumen and wall thickness measurements reflected significantly ($P<0.05$) higher values in adult dromedaries.

Microscopic parameters:

Histomorphometric parameters of dromedary's heart are shown in Table 3, Fig 2-3. Endocardium was seen significantly ($P<0.01$) thicker in adult than young dromedaries but the result ($P<0.01$) was otherwise for sub-endothelium thickness. Sub-endocardium thickness and purkinje fibres diameter was significantly ($P<0.01$) more in adult than young dromedaries. The statistical analysis revealed that percentage of parenchymal content in myocardium was significantly higher ($P<0.01$) in adult than young ones. However, the stromal content was significantly higher ($P<0.01$) in young than adult dromedaries.

The heart of the dromedaries' appeared reddish brown in colour having conical shape, markedly broad at the base that narrows in the middle and pointed at the apex. It was lodged with white coronary fat. These findings were in line with those described by Rehan and Qureshi (2007) in camel calves. The absolute weight was found more in adult (1.93 ± 0.09 kg) as compared to young (1.28 ± 0.10 kg) dromedaries. These findings are supported by

Table 1. Physiological status of young and adult camels (n=7).

Dromedaries Group	Body Scoring	Age (year)	Body Weight (kg)	Rectal Temp. (°F)	Respiration Rate (per min.)	Pulse Rate (per min.)
Young	2.5 to 3	1 to 2	180-250	101.4-102.2	13-22	48-63
Adult	2 to 3	>5	350-400	99.8-102.6	11-16	39-54

Nawal *et al* (2002) and Babiker (2004) who reported that weight of heart ranges from 2 to 5 and 1.1 to 1.2 kg in adult and young dromedaries, respectively. Similarly, Rehan and Qureshi (2007) determined absolute heart weight (1.14±0.05 kg) of camel calves which supported the current study finding that might be due to the fact that with advancing age and body weight, the absolute weight of heart increases in all species. The values of absolute heart weight in case of buffalo and cattle are 2.56 and 2.23 kg, respectively reported by Panhwar *et al* (2004) cited by Qureshi *et al* (2017). Relative weight of heart was 6.68±0.19 g/kg in young dromedaries that significantly (P<0.05) higher than adult weight 5.71±0.13 g/kg. No previous data was available to describe the relative heart weight of dromedaries. These statistical figures showed that relative weight of heart decreases with advancing age. No previous literature was found to compare this finding in camels. However, this phenomenon is supported by Hussain *et al* (2006) for buffalo's heart. Heart to body weight ratio in different animals reported by Lelovas *et al* (2014) as 5g/kg in humans, 7g/kg in adult dog and 3g/kg in adult pig and sheep.

The mean length (cm) of heart in young and adult dromedaries was 19.43±1.3 and 20.83±0.40 respectively with no significant (P>0.05) difference. Rehan and Qureshi (2007) estimated camel calf heart length of 19.54±0.44 cm which was in line with the findings of current study. However, no previous literature is available to describe the influence of age on heart length in dromedaries. The heart length was directly proportional to age in buffalo as described by Hussain *et al* (2006). Similar proportion of heart and age was documented by Shah *et al* (2010) in kids (7.38cm) and adult (7.75cm) of goats. Hence, the current findings of age influence on heart length do not support the previous findings in other species. However, circumference was measured significantly (P<0.05) more in adult dromedaries as compared to young ones which are in agreement with the observations of Hussain *et al* (2006) in buffalo and Shah *et al* (2010) in goats. It may be suggested from this outcome that a non-significant increase in heart

Table 2. Mean ± SEM of morphometric parameters of heart in young (1-2 year) and adult (>5 years) dromedaries.

Morphometric parameters		**Young**	**Adult**	**P-value**
Shape		Conical	Conical	-------
Colour of Coronary Fat		White	White	-------
Absolute Weight (kg)		1.28±0.10	1.93±0.09*	0.0330
Relative Weight (g/kg)		6.68±0.19**	5.71±0.13	0.0055
Length (cm)		19.43±1.3	20.83±0.40	0.2587
Circumference (cm)		33.4±0.58	35±0.46*	0.0103
Atrial Wall Thickness (cm)	Right	0.366±0.04	0.5±0.05	0.2873
	Left	0.41±0.04	0.63±0.08	0.2044
Ventricle Wall Thickness (cm)	Right	1.03±0.20	1.1±0.22	0.8399
	Left	1.66±0.24	2.76±0.05*	0.0486
Moderator Band	Width (cm)	0.93±0.06	2.06±0.21*	0.0305
	Thickness (cm)	0.6±0.23	0.56±0.03	0.9098
Aorta	Wall thickness (cm)	0.5±0.05	0.88±0.05*	0.0142
	Lumen Diameter (cm)	2.6±0.25	3.53±0.18*	0.0339

*= Significant at P<0.05; **= Highly significant at p<0.01

Table 3. Mean ± SEM of microscopic parameters of heart in young (1-2 year) and adult (>5 years) dromedaries.

Microscopic parameters		Young	Adult	P-value
Endocardium (µm)		172.2±4.2	218.4±5.6**	0.002
Sub Endothelium (µm)		96.1±8.1**	58.8±2.9	0.003
Sub Endocardium (µm)		78.3±2.8	111.1± 5.6**	0.001
Purkinje Fiber Diameter (µm)		40.6±2.2	52.7±1.9**	0.003
Myocardium	Parenchyma (%)	76.6±0.8	87.5±0.7**	0.006
	Stroma (%)	23.4±0.8**	12.3±0.6	0.004

*= Significant at P<0.05; **= Highly significant at P<0.01

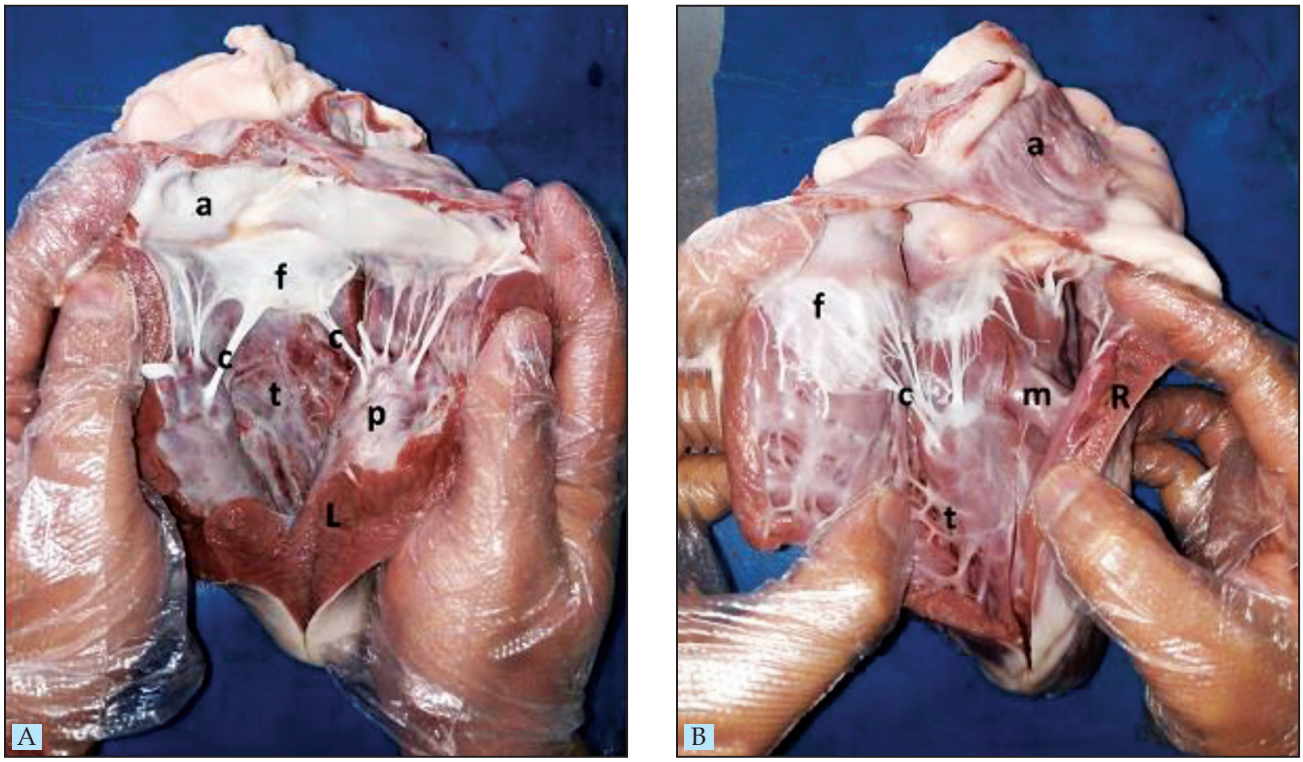

Fig 1. Pictorial view of left (A) and right side of heart: a; atrium, f; flap of semilunar cusp, c; chordae tendinae, p; papillary muscles, t; trabeculae carneae, L; left ventricular wall, R; right ventricular wall, m; moderator band.

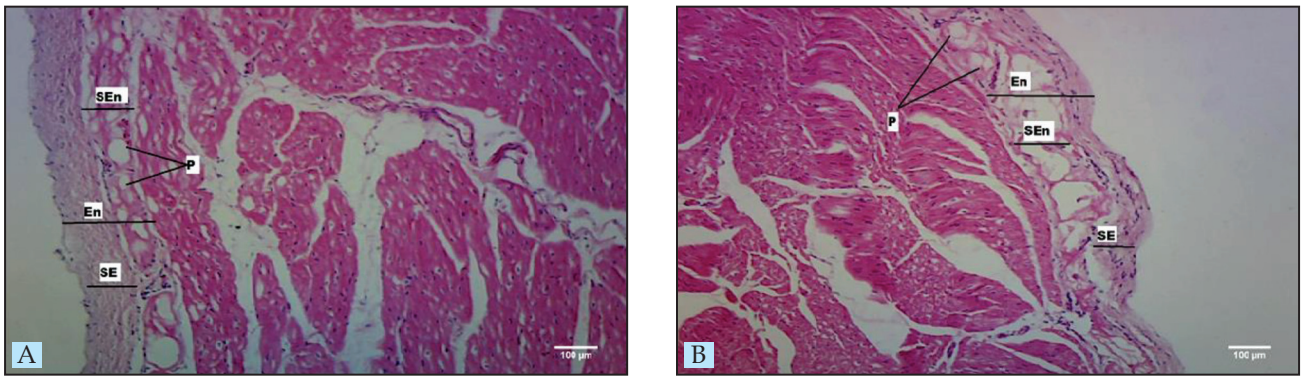

Fig 2. Cardiac histomicrograph of Young (A) and Adult (B) dromedaries (H&E 100X): Histological parameters i.e thickness of endocardium (En), Sub-endocardium (SEn) and diameter of purkinje fibre (P) is significantly (P<0.05) higher in adult dromedaries while sub-endothelium (SE) of young dromedaries is significantly (P<0.05) thicker.

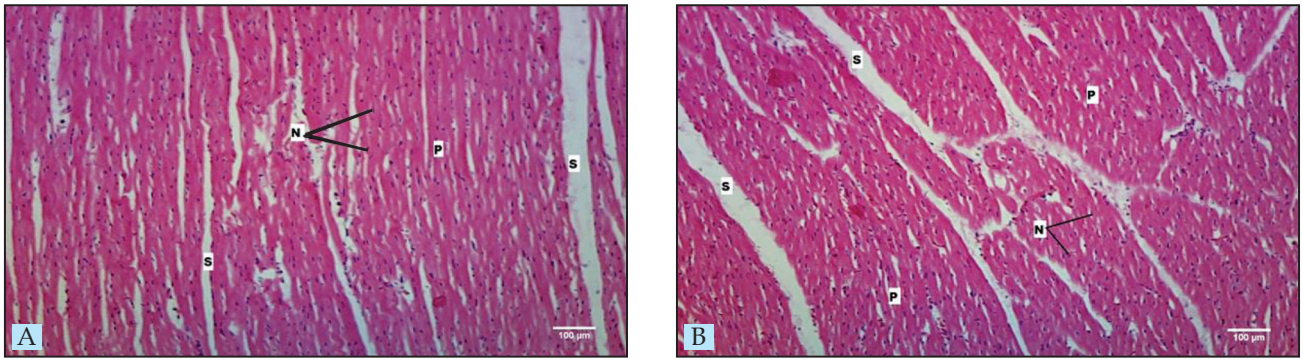

Fig 3. Cardiac histomicrograph of Young (A) and Adult (B) dromedaries (H&E 100X): Percentage of stromal content (S) is significantly (P<0.05) higher in young dromedaries while Parenchymal (P) percentage is found significantly (P<0.05) higher in adult dromedaries.

length may be accommodated for its significant increase in circumference. Age influence was witnessed non-significant (P>0.05) in case of right and left atrium, and right ventricle wall thickness while the left ventricle wall thickness was significantly (P<0.05) higher in adult camels as left ventricle requires more pressure for adequate blood circulation in the larger body. These results are in accordance to Tharwat *et al* (2012) results; they estimated the wall thickness (cm) of right and left ventricle as 1.5±0.2 and 2.8±0.7, respectively using echocardiography in adult dromedaries. The mean values of moderator band width and thickness depicted that width had significantly (P<0.05) increasing trend with increasing age while the later had remained unchanged with increasing age. This may be due to the fact that in adult dromedaries heart has to pump with more stroke volume, hence, a stronger moderator band is required to protect the overexpansion of left ventricle muscular wall. No anatomical statistics are available to compare these findings in animal species but mean thickness in adult human heart is reported 4.5±1.8 mm (Loukas *et al*, 2010). The luminal diameter and wall thickness of aorta at the base of heart were observed significantly (P<0.05) more in adult dromedaries. The age dependent literature is unavailable on these parameters of camel but Tharwat *et al* (2012) estimated the diameter of both atria and ventricles along with aortic diameter using echocardiography of adult dromedaries' heart that supported current findings of adult aortic parameters. This may be due to the fact that with advancing age and body weight thickening of these organs occur.

Microanatomical data revealed that thickness of endocardium and subendocardium layers were significantly (P<0.01) more in adult as compared to young dromedaries while subendothelium thickness followed the reverse trend. This can be linked with the higher absolute heart weight in adult dromedaries. The diameter of purkinje fibres was also seen significantly (P<0.01) increased in adult dromedaries as they require expanded conduction system for cardiac action. The parenchymal content (%) in myocardium of adult dromedaries was significantly (P<0.01) higher than young ones', while this pattern was observed inversed in case of stromal content (%). Rehan and Qureshi (2006) described the connective tissue percentage of right and left ventricle 7.26±0.28 and 6.8±0.32, respectively, in camel calves. In buffaloes, the connective tissues percentage in right and left ventricles was reported as 2.86 and 2.2, respectively (Panhwar *et al*, 2004). Age dependent intramural connective tissue in teddy goats reported by Qureshi *et al* (2013) showed a positive trend from young to adult followed by a negative trend from adult to old goats which is not in agreement to camel heart. The comprehensive age dependent data of microanatomy of camel heart is yet to be known to compare other microscopic structural findings.

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