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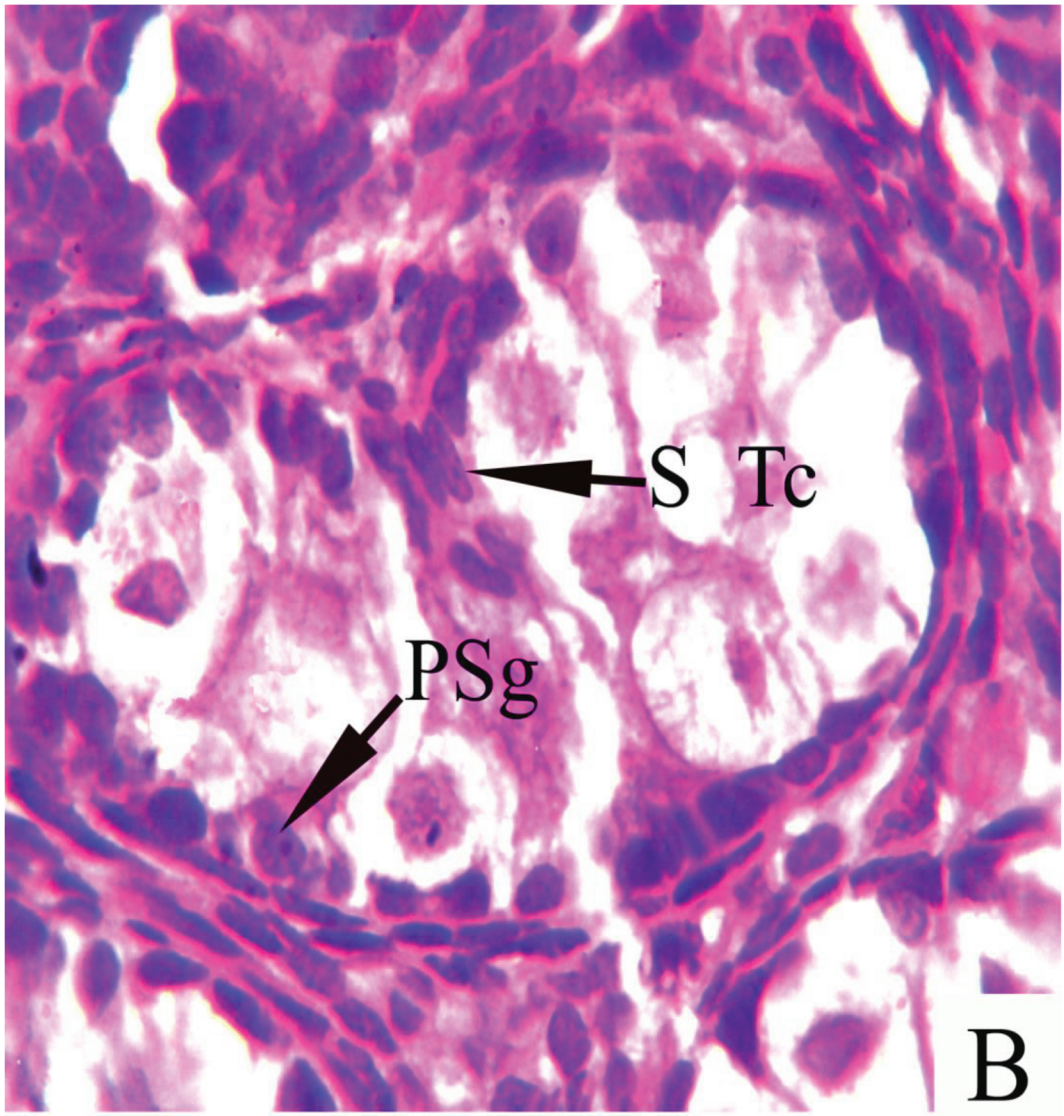

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Contents

Volume 11 **April 2018** **Number 1**

Curcumin (*Curcuma longa*) Protects Against the Adverse Effect of Long Term Administration of Lithium on Cerebral and Cerebellar Cortices in Rats Histological and Immunohistochemical Study

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Abstract

Administration of lithium, antidepressant and psychiatric medication, is always prolonged. This study was aimed to detect the adverse effects of long term administration of lithium on cerebral and cerebellar cortices in rats in addition to assess the possible protective effect of curcumin using histological and immunohistochemical methods. Rats were divided into 3 groups (10 for each); group I (control) given distilled water and DMSO orally, group II received lithium carbonate dissolved in distilled water (150 mg/ kg b. wt. / day / intragastric), and group III received curcumin dissolved in 50% DMSO (200 mg/ kg b. wt. / day/ intragastric) 1 hr before lithium carbonate administration for 6 weeks. We examined the cerebrum and cerebellum of rats for glial reactions and cell proliferation by using immunolabelling for glial fibrillary acidic protein (GFAP) and Ki67, respectively. In lithium treated group, both cerebral and cerebellar cortices showed an increased number of positive glial cells for GFAP that was decreased in curcumin treated group. For ki67, cerebral and cerebellar cortices of both lithium and curcumin treated groups showed an increased number of ki67 immunopositive cells. This study advises to administrate curcumin in concomitant with lithium therapy as it can protect against lithium neurotoxicity.

Introduction

Depression is a very common and worldwide disease (Aakhus et al., 2012). Although the efficacy of anti-

depressant drugs and decades of use, their side effects and how toavoid them is still under continuous investigations.

Lithium is a potent mood stabilizer (Zanni et al., 2017) therefore; it is one of the most widely used antidepressant and psychiatric medication (Sharma and Iqbal, 2005). In addition, it has an anti-suicide (Cerqueira et al., 2008) and anticonvulsant effects (Ahmed, 2013). Since the therapy is usually prolonged, it is unlikely to be without complications or side effects on the brain (Csutora et al., 2005) and other organs like kidney and heart (Sharma and Iqbal, 2005; Shah et al., 2015).

Medicines derived from plants play a pivotal role in the health care of many cultures. Curcumin is a yellow to gold colored spice that has been derived from the root turmeric plant Curcuma longa (Nabiuni et al., 2011). Curcumin is commonly known as antioxidant and anti-inflammatory (Aggarwal et al., 2007). Also, Curcumin has been described as a neuroprotective agent (Nabiuni et al., 2011) as well as its administration significantly control brain injury (Thiyagarajan and Sharma, 2004).

Since neurotoxicity has been assessed depending on classical histological observations (Gross and Kramer, 2003), the current study aimed to assess the possible protective effect of curcumin against lithium induced cerebral and cerebellar toxicity in adult rat using histological and immunohistochemical methods. Glial fibrillary acidic protein (GFAP), an intermediate filament protein of astrocyte, has been serving as a neurotoxicity biomarker (O'Callaghan and Sriram, 2005). The presence of ki-67 protein during all active phases of the cell cycle (G1, S, G2, and mitosis) makes it an excellent marker for cell proliferation (Scholzen and Gerdes, 2000).

Materials and Methods

Animals

Thirty adult male Albino rats (200-220 g body weight) were purchased from lab animal house, Faculty of Veterinary Medicine, Benha University, Egypt to be used for this study. The rats were kept for 10 days before the experiment under good hygienic condition at room temperature, and were fed standard diet and watered ad libitum.

Chemicals

- **Lithium carbonate**

Tablets of Prianil CR (Nile Company for Pharmaceuticals and Chemical industries, Cairo, Egypt) were used as a source of lithium. Each tablet contains 400 mg of lithium carbonate.

- **Curcumin**

Curcumin powder was purchased from Sigma, Cairo, Egypt.

Experimental design

The experiment followed the guidelines of Ethical Committee of Benha University. The rats were divided into 3 groups, each of 10 rats as follow:

Group I (control group): Rats were given the same amount of vehicle (distilled water and DMSO) orally for 6 weeks.

Group II: Rats received toxic dose of lithium carbonate dissolved in distilled water (150 mg/kg b.wt./ day/ intragastric according to Vijaimohan et al., 2010) for 6 weeks.

Group III: Rats in this group received curcumin dissolved in 50% DMSO (200 mg/kg b.wt/ day/ intragastric) according to Ahmed (2013) 1 hr before the administration of the same dose of lithium carbonate as group II daily for 6 weeks.

Tissue collection and processing:

Twenty-four hrs. after the last dose, all rats were anaesthetized with ether inhalation and decapitated. Skull of each rat was opened and the brain was removed carefully. Mid sagittal section of the brain was obtained then immersed in 10% neutral buffered formalin for 48 hrs. Routine histological work was done to obtain paraffin blocks.

Histological examination

Five µm thickness paraffin sections were collected, deparaffinized and rehydrated using the standard techniques according to Bancroft and Gamble (2007). Paraffin sections were stained with hematoxylin and eosin (H&E) for general structure and assessment of histological changes.

Immunohistochemical examination

Paraffin sections were collected into positive slides and processed for immunohistochemical examination using an avidin biotin peroxidase method according to Kiernan (2008). Deparaffinization and hydration were done before antigen retrieval which was performed by heating the slides in citrate buffer (pH 6.0) for 10 min in a steamer. To block endogenous peroxidase activity, slides were dipped in absolute methanol containing 3% (v/v) hydrogen peroxide for 10 min at RT. Sections were then incubated overnight at 4ºC with monoclonal mouse anti-ki67 (clone MM1, Novocastra Laboratories Ltd, UK) at 1:100 dilution and monoclonal anti- GFAP (AM020-5M Bio-genex) at 1:5000 dilution. Next, sections were exposed to biotinylated secondary antibody (Dako, USA) diluted 1:200 for 30 min at room temperature. Visualization was done using commercial peroxidase streptavidin complex (ABC; Dako, USA) for 30 min then 3,3´-diaminobenzidine tetrahydrocholoride (DAB; Dako, USA) for 2 min at room temperature. Finally, the sections were counterstained with hematoxylin. Negative control sections were incubated with normal

goat or rabbit serum instead of the primary antibodies (Dako, USA).

Results

Group I (control group)

In H&E stained sections, normal histological appearance of both cerebrum and cerebellum was identified (Figs. 1A, 2A). Cerebral cortex of control group showed a clear pia mater, followed by a molecular layer then different pyramidal cell layers (Fig. 1A). The latter consist of nerve cells of various sizes and shapes and with vesicular nuclei (Fig. 1D).

Cerebellar cortex was formed of outer molecular, Purkinje cell layer and inner granular layers (Fig. 2A). The molecular layer is formed of scattered cells and nerve fibers. The Purkinje cell layer was the middle layer and consisted of large pyriform cells with clear vesicular nuclei arranged in one row along the upper margin of the granular layer. The granular layer was the inner most layer of the cerebellar cortex and composed of tightly packed small rounded cells with deeply stained nuclei (Fig. 2D).

Immunohistochemical staining of cerebral cortex for GFAP showed positive immunostaining in star shaped glial cells and their processes (Fig. 3A). Also, glial cells in molecular and granular layers of cerebellum showed positive immunostaining for GFAP (Fig. 3D).

For ki67 immunostaining, some cells in cerebral cortex showed nuclear immunostaining for ki67 (Fig. 4A) in addition to, cerebellar cortex showed positive nuclear immunestaining in some cells of granular layer close to the molecular layer while Purkinje cells were not immunoreactive (Fig. 4D).

Group II (lithium treated group)

Congestion and haemorrhage were observed in the blood vessels of the cerebral meninges (Fig. 1B). Marked degeneration of neurons with pyknotic nuclei, vacuolar spaces around the pyramidal cells and a pronounced interstitial edema were commonly detected (Fig. 1E). Also, haemorrhage in the blood vessels of the cerebellar meninges could be seen (Fig. 2B). Purkinje cells appeared either degenerated with shrinkage of their cytoplasm and pyknotic or vacuolated nuclei (Fig. 2E).

There were an increased number of the positive GFAP immunostaining glial cells in cerebral cortex (Fig. 3 B) cerebellum (Fig. 3 E) and that appeared star shaped cells with increased branches of their cytoplasmic processes. The molecular layer of cerebellum showed an increased GFAP immunostaining in the Bergmann glia (modified astrocytes) which appeared perpendicular to the pial surface and parallel to each

other (Fig. 3 E). For ki67 Immunostaining, cerebral cortex showed more positive cells and intense staining with mitotic figures (Fig. 4B). Also, cerebellar cortex revealed stronger immunostaining and more positive cells (Fig. 4E) in comparison to control.

Group III (protective group)

The cerebral cortex of the treated group showed an improvement in their histological structure with slight congestion of blood vessels (Fig. 1C). Although slight edema and vacuolation were still present (Fig. 1F). Cerebellum of this group showed slight congestion of meningeal blood vessels and medullary capillary (Fig. 2C). Most of Purkinje cell layer appeared same as normal cells (Fig. 2F).

Immunohistochemical staining of cerebral (Fig. 3C) and cerebellar (Fig. 3F) cortices showed fewer GFAP positive cells with their processes nearly similar to the control group, especially those in the granular layer. For ki67, both cerebral and cerebellar cortices showed nearly the same feature of lithium treated group where more ki67 positive cells were seen (Figs. 4C and 4F, respectively) in comparison to control.

Discussion

The current work revealed normal histological structure of the cerebral and cerebellar cortices of the control rats as that stated by Mescher (2015). Histological observations of the cerebral cortex of lithium treated rats showed degenerated neurons with vacuolation indicating brain damage as reported by Young (2009) after prolonged lithium intoxication. Also, the cerebellar cortex of lithium treated rats showed distorted and degenerated Purkinje cells and vacuolation, in some areas indicating cell loss. This finding was in agreement with Cerqueira et al., (2008) and Bashandy (2013). Additionally, the observed haemorrhage of meningeal blood vessels was similar to Loghin et al., (1999) however Bashandy (2013) reported congestion of blood vessels.

Astrocytes, star-shaped glial cells in the central nervous system, have a major role in supporting neurons, scar formation and maintenance of the blood brain barrier (Mescher, 2015). Our immunohistochemical findings revealed positive GFAP immunostaining in the cytoplasm and processes of the astrocytes in the cerebrum and cerebellum of control rats that was in agreement with Bashandy (2013). In lithium treated group, an increase in number of strongly GFAP immunestained glial cells (gliosis) and their processes was similar to that described by Halliday et al. (1996) and Bashandy (2013). The increased

number of glial cells may be a compensatory response to brain injury and neuronal degeneration caused by lithium. Ibrahim et al. (2015) demarcated that lithium induced noticeable degeneration of neurons and demylination of nerve fibers in cerebrum.

The increased GFAP staining in Bergmann glia of the cerebellar molecular layer was similar to the finding of Wagemann et al. (1995). The strong GFAP immunostaining with increased cell processes in the astrocytes of granular layer was similar to Hashish (2014).

For ki67, the cells of both cerebral and cerebellar cortices of control group showed immunoreactivity. These cells increased in number and immunostaining intensity in lithium treated group. This finding was in agreement with Zanni et al. (2017), who owed this finding to the positive effects of lithium on neurogenesis; generation and integration of new neurons.

Histological examination of the curcumin protected group showed structural improvement of the cerebral and cerebellar cortices in comparison to lithium treated group, suggesting the neuroprotective property of curcumin (Nabiuni et al., 2011; Nasir and Jaffat, 2016). Immunohistochemical finding of the curcumin protected group showed decreased GFAP immune-staining compared to lithium treated group. This finding was in harmony with Parastoo et al. (2015), who detected significant decrease of GFAP in curcumin group following acute spinal cord injury, suggesting that curcumin can limit gilosis. On the other hand for ki67, curcumin protected group showed nearly the same feature of lithtium treated group where more ki67 positive cells were detected. This indicates that curcumin has stimulating effect on neurogenesis, likewise lithium (Xua et al., 2007; Attari et al., 2016).

Conclusion

The overdose and/or chronic therapy with lithium has neurotoxic effect on the cerebrum and cerebellum characterized by sever alteration in brain ultrastructure. This study advises to use curcumin co-treatment with lithium therapy due to its ameliorating properties against drug neurotoxicity.

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Fig.1

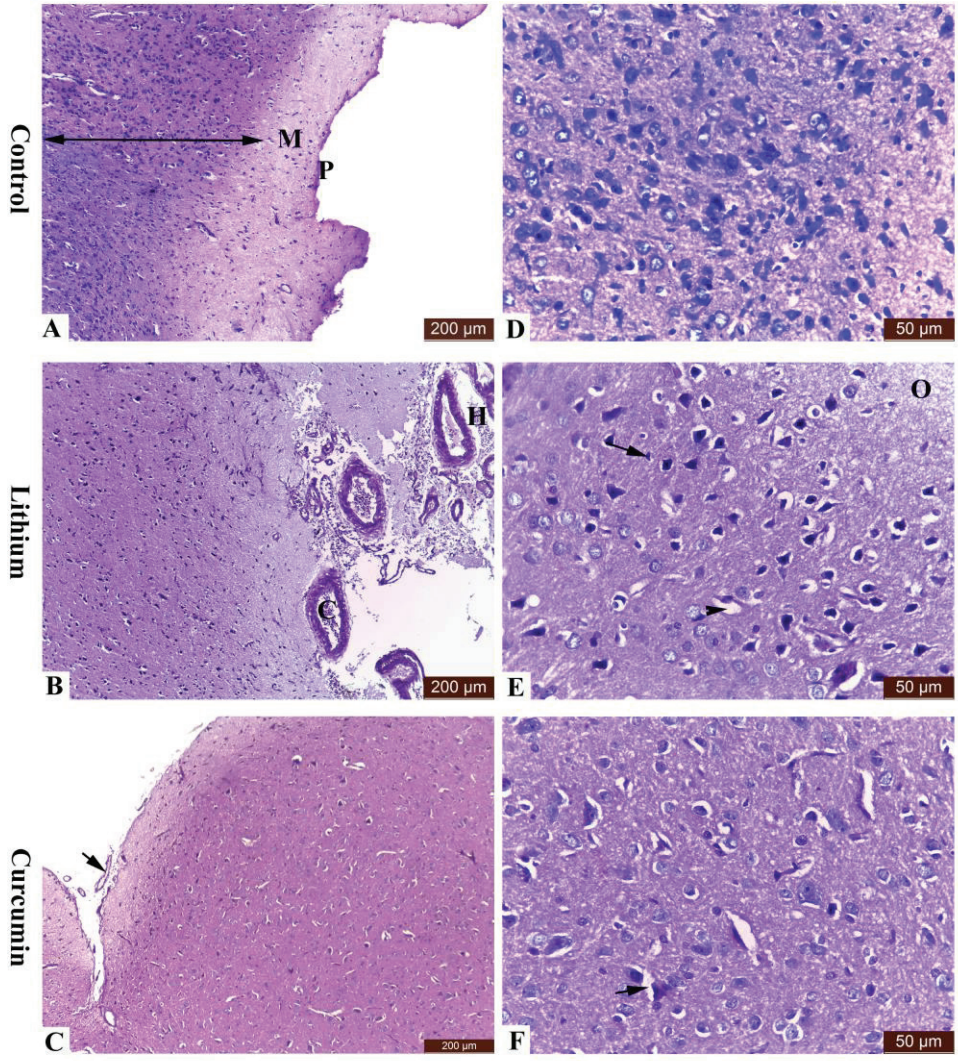

Fig (1): Photomicrograph of cerebral cortices of control (A, D), lithium treated (B, E) and curcumin treated (C, F) rats. A: Normal histological structure of cerebral cortex. P, pia mater; M, molecular layer;↕, pyramidal cells layers. B: Showing congestion (C) and haemorrhage (H) in the blood vessels of the meninges. C: Showing slight congestion of blood vessels (arrow). D: Showing different sizes and shapes of pyramidal cells with vesicular nuclei. E: Showing degenerated neurons with pyknotic nuclei (arrow), vacuolar spaces around the pyramidal cells (head arrow), and a pronounced interstitial oedema (O). F: Showing slight edema and vacuolation (arrow). Scale bars (A-C) = 200 µm and (D-F) = 50 µm.

Fig.2

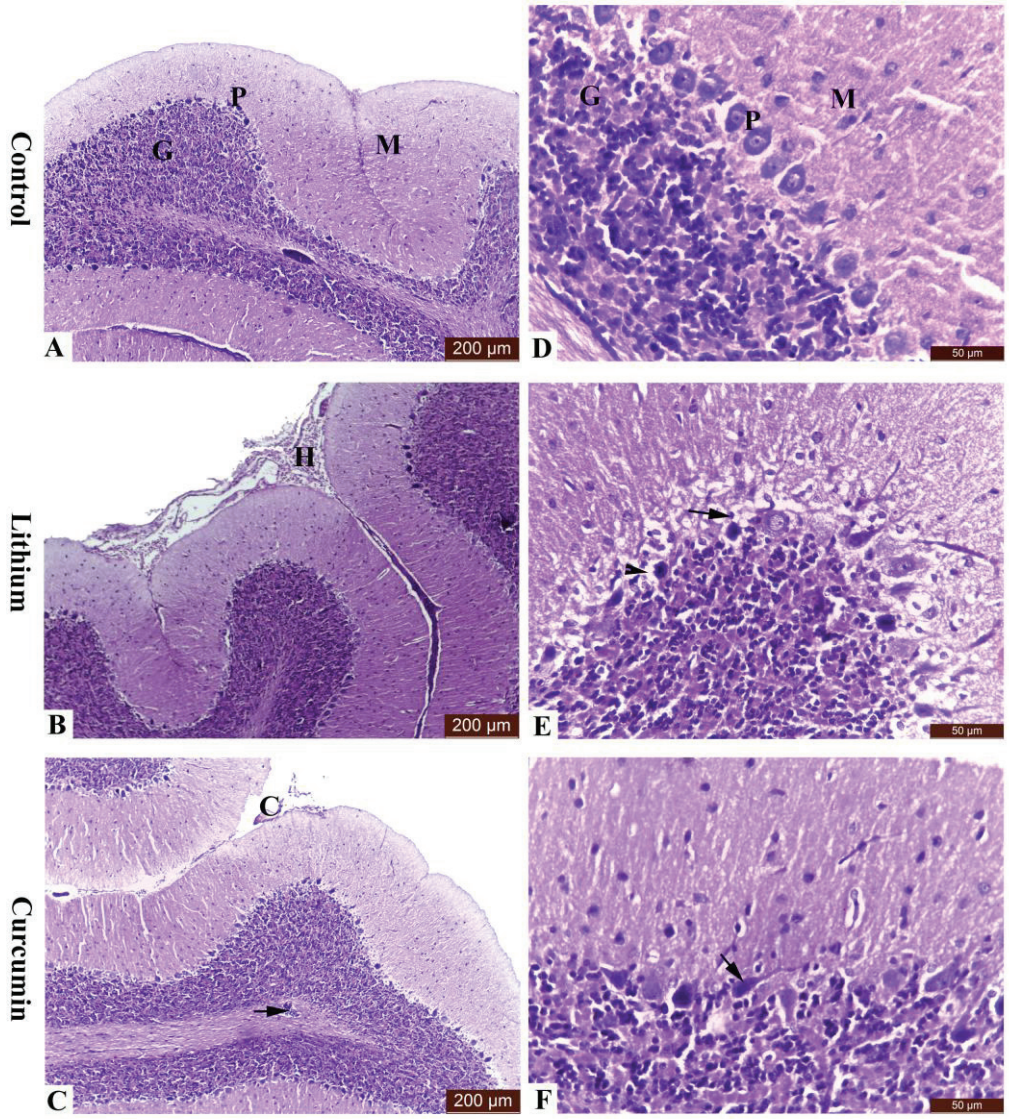

Fig (2): Photomicrograph of cerebellar cortices of control (A, D), lithium treated (B, E) and curcumin treated (C, F) rats. A: Normal histological structure of cerebellar cortex. M, molecular layer; P, Purkinje cells; G, granular layer. B: Showing haemorrhage at the cerebellar meninges (H). C: Showing slight congestion (C) of meningeal blood vessels with slightly congested medullary capillary (arrow). D: Higher magnification of A. M, molecular layer; P, Purkinje cells; G, granular layer. E: Showing Purkinje cells appeared either degenerated with shrinkage of their cytoplasm and pyknotic nuclei (arrow) or vacuolated indicating cell loss (head arrow). F: Showing normality of most of Purkinje cell layer (arrow). Scale bars (A-C) = 200 µm and (D-F) = 50 µm.

Fig.3

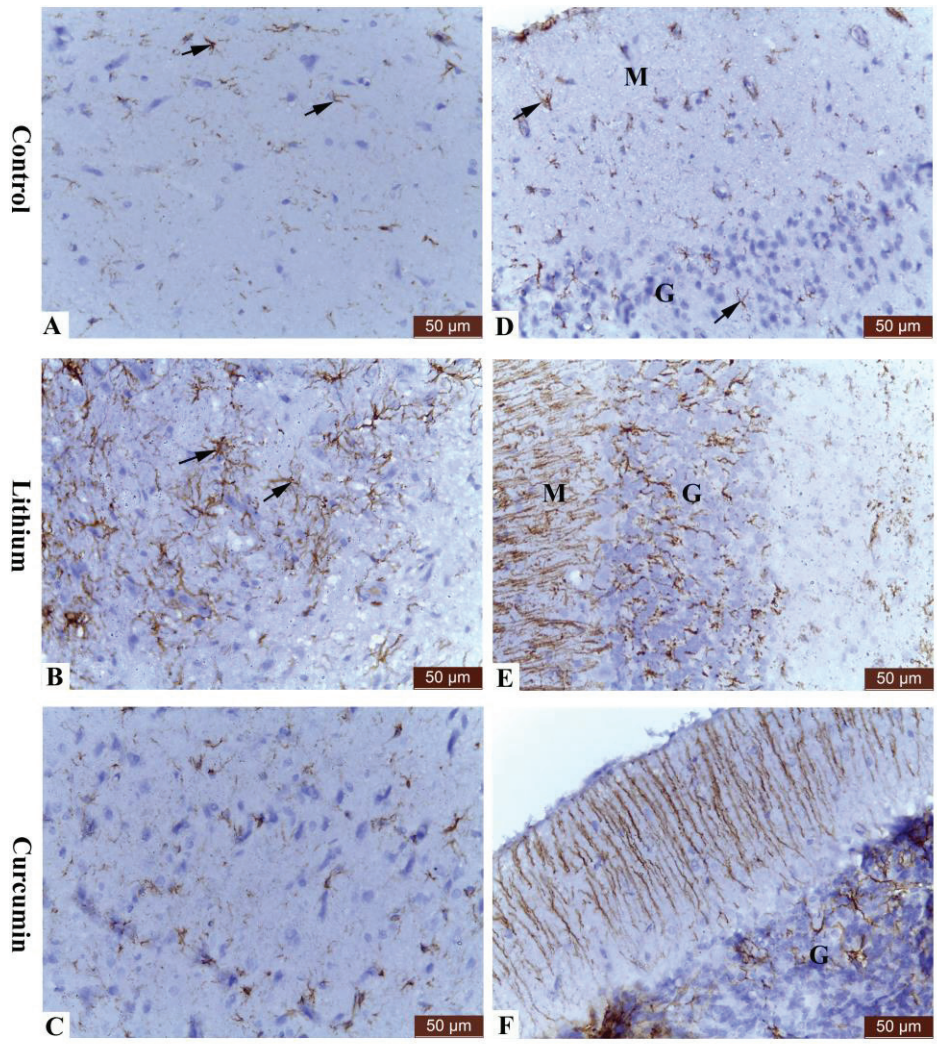

Fig (3): Photomicrograph of GFAP immunostainings in cerebral and cerebellar cortices of control (A, D), lithium treated (B, E) and curcumin treated (C, F) rats. A: Cerebral cortex showing positive immunostaining in star shaped glial cells and their processes (arrows). B: Cerebral cortex showed increased number of the positive glial cells with increased branches of their cytoplasmic processes (arrows). C: Cerebral cortex showed fewer positive cells. D: Cerebellar cortex showing positive immunostaining in glial cells (arrows) of molecular (M) and granular layers (G). E: Cerebellar cortex showed strong and increased immunopositive Bergmann glia of the molecular layer (M) and star shaped glial cells and their processes of the granular layer (G) than D. F: Cerebellar cortex showing fewer positive glial cells especially those in granular layer (G) than E. Scale bars = 50 µm.

Fig.4

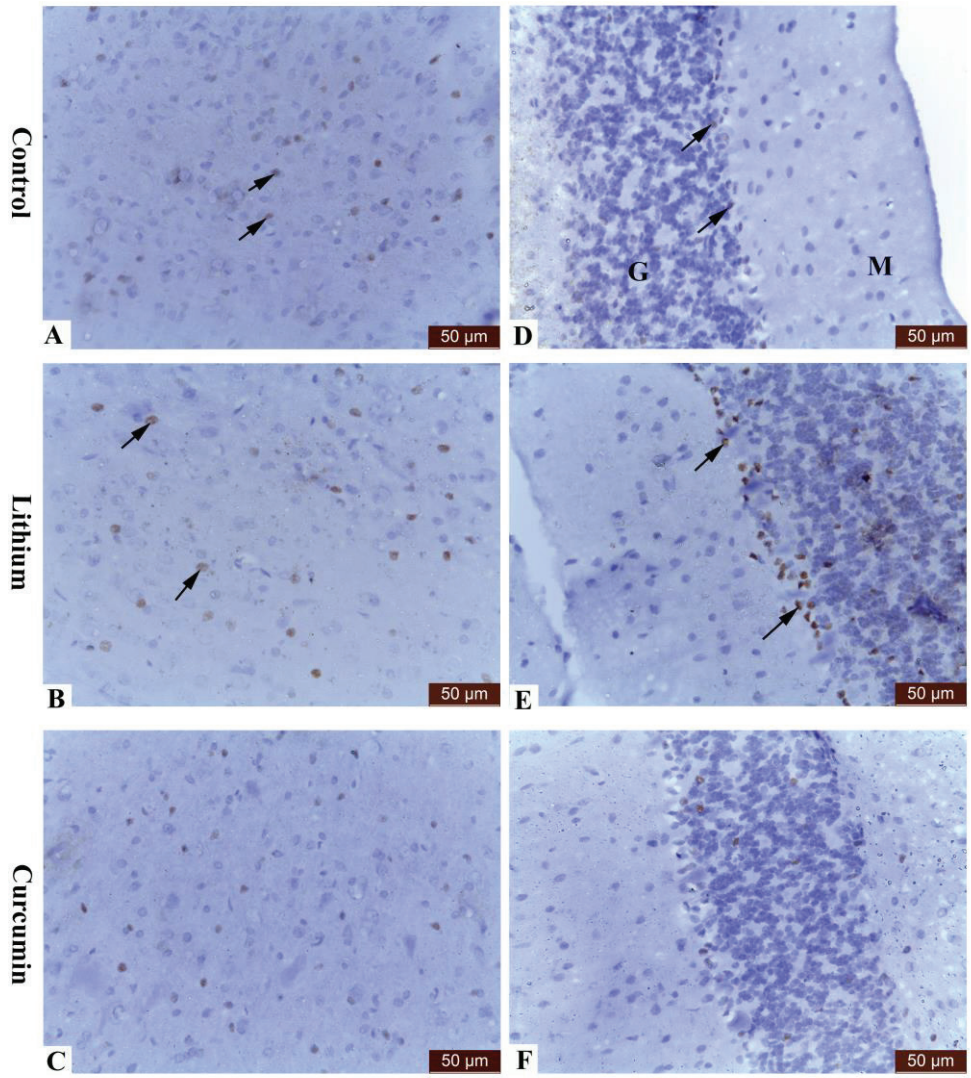

Fig (4): Photomicrograph of ki67 immunostainings in cerebral and cerebellar cortices of control (A, D), lithium treated (B, E) and curcumin treated (C, F) rats. A: Cerebral cortex showed some positive cells (arrows). B: Cerebral cortex showed more positive cells and increased staining intensity with mitotic figures (arrows). C: Cerebral cortex showed nearly the same feature of B. D: Cerebellar cortex showed some positive cells (arrows) of granular layer (G) close to the molecular layer (M). E: Cerebellar cortex showed strong immunostaining and more positive cells (arrows). F: Cerebellar cortex showed nearly the same feature of E. Scale bars = 50 µm.

Animal species in this issue

Rat (*Rattus rattus*)

Kingdom: Animalia & Phylum: Chordat & Class: Mammalia & Order: Rodentia & Superfamily: Muroidea & Family: Muridae & Subfamily: Murinae & Genus: ***Rattus*** & Species: *50 species*

Rats are various medium-sized, long-tailed rodents of the superfamily Muroidea. "True rats" are members of the genus *Rattus*, the most important of which to humans are the black rat, ***Rattus rattus***, and the brown rat, ***Rattus norvegicus***. Many members of other rodent genera and families are also called rats and share many characteristics with true rats.

Rats are typically distinguished from mice by their size; rats are generally large muroid rodents, while mice are generally small muroid rodents. The muroid family is very large and complex, and the common terms *rat* and *mouse* are not taxonomically specific. Generally, when someone discovers a large muroid, its common name includes the term *rat*, while if it is small, the name includes the term *mouse* - scientifically, the terms are not confined to members of the *Rattus* and *Mus* genera.

The best-known rat species are the Black Rat (*Rattus rattus*) and the Brown Rat (*Rattus norvegicus*). The group is generally known as the Old World rats or true rats, and originated in Asia. Rats are bigger than most Old World mice, which are their relatives, but seldom weigh over 500 grams (1 lb) in the wild.

The term "rat" is also used in the names of other small mammals which are not true rats. Examples include the North American pack rats, a number of species loosely called kangaroo rats, and others. Rats such as the Bandicoot rat (*Bandicota bengalensis*) are murine rodents related to true rats, but are not members of the genus *Rattus*. Male rats are called *bucks*, unmated females are called *does*, pregnant or parent females are called *dams*, and infants are called *kittens* or *pups*. A group of rats is either referred to as a *pack* or a *mischief*.

Expression Pattern of DMRT1 and STRA8 Genes During Postnatal Development of Rabbit Testes

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With 2 figs, 2 tables Received April 2017, accepted for publication March 2018

Abstract

Thirty New Zealand rabbits of various ages were used for this investigation. DMRT1 and STRA8 genes expression was recorded by RT PCR, completed by histological sections of testes in studied ages. DMRT1 and STRA8 genes have distinct roles during spermatogenesis in mammals. The present study hypothesized that the change in the level of specific genes expression during spermatogenesis could be related to the change in the complement of germ cell types. Distinct expression patterns were observed for DMRT1 reached the maximum level (peak or plateau) just before spermatogenesis process at age of 6.5th week and testicular STRA8 reached the maximum level at 6 months. The two genes expression patterns are consistent with their specific roles during spermatogenesis. The purpose of the present study was to determine the expression of DMRT1 and STRA8 genes throughout postnatal New Zealand rabbit testis that would serve as a reference for expression pattern of the studied genes.

Keywords: expression, testes, DMRT1, STRA8, postnatal, spermatogenesis, rabbit.

Introduction

The rabbit is an excellent model used in a variety of biomedical research fields including neuroscience, oncology, embryonic development, cardiovascular studies, dermatology, and reproduction (Ewuola & Equnike, 2010; Asano *et al*., 2011 and Guo *et al*., 2012). Rabbit is an attractive species for making gonad studies due to easily identification of the morphological changes of the seminiferous epithelium cycle (Ewuola and Equnike, 2010). The period of

pre-spermatogenesis exceeds that of other laboratory animals such as rat. This particular characteristic ma-kes the development of rabbit closer to that of human (Wu *et al*., 2003; Vigueras *et al*., 2006 and Culty, 2009).

Spermatogenesis is a complexly regulated process and any genetic disturbance in it leads to male infertility. The adult mammalian testis is among the body's most proliferative tissues, producing millions of highly specialized gametes, or spermatozoa each day. Spermatogenesis is carefully regulated, ensuring that spermatozoa are produced at a constant rate (Endo *et al*., 2015).

Double-sex and mab-3-related transcription factor1 (DMRT1) acts as the 'gatekeeper' that prevents uncontrolled entry of spermatogonia to meiosis (Don *et al*., 2011). DMRT1 is required for spermatogenesis as it is critical to maintain the balance between mitotic and meiotic germ cells. In addition, it has a prominent role in testicular differentiation in all vertebrates (Zarkower, 2012). DMRT1 is essential in spermatogonia to restrict retinoic acid responsiveness and directly repress STRA8 transcription, thereby preventing meiosis and promoting spermatogonial development by coordinating spermatogonial development and mitotic amplification with meiosis, as a result, DMRT1 allows abundant and continuous production of sperms (Matson *et al*., 2010). In mice, DMRT1 is expressed in all mitotic spermatogonia, but the expression decreases with the onset of spermatogonial differentiation and disappears at the initiation of meiosis (Matson *et al*., 2010). Moreover, Don *et al*. (2011) stated that DMRT1 is highly expressed in undifferentiated spermatogonia, less abundantly expressed differentiating spermatogonia, and absent from preleptotene spermatocytes or other meiotic or postmeiotic cells.

Stimulated by retinoic acid gene 8 (STRA8) protein plays roles for the progression of meiosis (Choi *et al*., 2010). Additionally, the gene STRA8 is essential for meiotic initiation, also promotes (but is not required for) spermatogonial differentiation (Endo *et al*., 2015). In mice, STRA8 expression begins immediately before spermatogonial differentiation as well as STRA8 expression begins in late undifferentiated spermatogonia and persists in differentiating spermatogonia (Endo *et al*., 2015). Additionally, STRA8 expression is increased in preleptotene spermatocytes (premeiotic cells) as well as in undifferentiated spermatogonia (Zhou *et al*., 2008 and Mark *et al*., 2015). Because STRA8 promotes spermatogonial differentiation and is required for

meiotic initiation, precisely timed increases in STRA8 expression might coordinate both transitions, ensuring their co-occurrence in stages of spermatogonial differentiation and meiotic initiation (Endo *et al*., 2015). To the best of our knowledge, this is the initial reference to analyze the expression pattern of rabbit DMRT1 and STRA8 genes during cellular development, using spermatogenesis as a model system.

Materials and Methods

Animals

The current study was carried out on 30 male New Zealand rabbits of various ages (0 dpp, 2w, 4w, 6w, 6.5w, 7w, 7.5w, 3m ,4m & 6m), three animals for each age were used to obtain their testes.

Histology

The animals were slaughtered according to guidelines of animal care committee of Faculty of Veterinary Medicine, Benha University. Testes were obtained from rabbits, fixed in Bouin's solution then dehydrated in graded ethanol. Tissues were then infiltrated and embedded in paraffin. Five micron sections were mounted onto gelatin-coated slides and stained with Haematoxylin & Eosin for general histological structures and periodic acid Schiff (PAS) for mucopolysaccharide using standard methods (Drury and Walington, 1980).

Quantitative RT-PCR

Rabbit testicular tissues were obtained and total RNA was extracted using total RNA Purification Kit following the manufacturer protocol (iNtRON Biotechnology, easy-REDTM Total RNA Extraction Kit). cDNA was synthesized from 5 µg of total RNA using M-MuLV Reverse Transcriptase enzyme following the manufacturer protocol (Thermo Scientific, Fermentas, # EP0451). Quantitative PCR was performed using 2X Maxima SYBR Green/ ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221). The results were analyzed by the delta-delta CT method using H2AFx gene as an internal reference. Results were plotted as percentage of maximum expression+/- SE.

RT-PCR primer sequences were as following:

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)
DMRT1	GGAGCCTCCCAGCACCTTAC	TGCATCCT GTACTGC GAACTCA
STRA8	GACAACAATGAGGCTCCAAATG	TGATCTG-CAC-GTAGAGC TGAAAC
H2AFx	ACCTGACGGCCGAGATCCT	CGCCCAG-CAGCTT-GTTGAG

Results

Histological observations:

Histological analysis of (0dpp-4weeks) old rabbit testes revealed that the testicular cords contained two types of cell populations, a large number of dark polygonal cells with irregular nuclei, Sertoli cells, and a small number of large, light, round cells with relatively round nuclei, pre-spermatogonia or undifferentiated spermatogonia (Figs.1 A, B & C). Toward the end of the period of pre-spermatogenesis (6 - 6.5weeks), germ cells appeared large with pale cytoplasm and large vesicular nuclei with clear nucleoli, late undifferentiated spermatogonia (Figs.1D, E).

At 7-7.5weeks old rabbit testis, spermatogenesis began at which the late undifferentiated spermatogonia underwent maturation and transformed to spermatogonia. The latter were spherical or ovoid with clear cytoplasm and large round nuclei with condensed chromatin island peripherally seated and their nuclei were eccentric or central and vesicular (Fig.1F,2B).

Histological examination of 3 months-old rabbit testis revealed the appearance of primary spermatocytes in some seminiferous tubules (Fig.2B). Spermatids were first seen at 4-months- old rabbit testis that reacted positively to PAS stain (Fig.2C). All stages of spermatogenesis were evident at 6-months old rabbit and spermatozoa appeared in the tubular lumen at this stage (Fig.2D).

Analysis of Gene expression:

The data obtained from RT- PCR revealed a significant ($P \leq 0.05$) gradual increase in the expression level of the DMRT1 gene in testis of rabbit from the day of the birth (0dpp) till reach the maximum level (peak or plateau) at age of 6.5W (Fig. 3). Following 6.5W, the expression declined gradually till reached the age of 3M. After that, the expression became very low but still present. The highest significant up-regulation was at age of 6.5W, while the lowest down-regulation was seen at age 6M.

The data obtained from RT- PCR revealed a significant ($P \leq 0.05$) gradual increase in the expression level of the STRA8 gene in testis of rabbit from the day of birth (0D) till reached the maximum level (peak or plateau) at age of 6M (Fig. 4). Unlike DMRT1 gene, the highest significant up-regulation in STRA8 gene was noticed at age from 3M to 6M, while the lowest down-regulation was seen at age from 0dpp to 6W.

Discussion

Previous studies showed strong correlation between the two genes

(DMRT1, STRA8) and spermatogenesis (Kim *et al.,* 2007; Mark *et al*., 2008; Choi *et al*., 2010; Maston *et al*., 2010; Don *et al.*, 2011 and Endo *et al*., 2015).

The current investigation showed the expression analysis at histo-logical level as gene expression was associated with precise stages of the seminiferous epithelium cycle. To begin our survey of DMRT1 and STRA8 expression in rabbit testis, we used RT-PCR to investigate the level of mRNA production for the two genes in total testis samples from rabbit at various ages (0 dpp, 2w, 4w, 6w, 6.5w, 7w, 7.5w, 3m, 4m & 6m).

The present work analyzed DMRT1 expression level which was gradually increased immediately after birth (0day) to (4w) which might be due to gradual increase in mitosis, which suggested that DMRT1 may play role in mitotic process. This was in accordance with Kim *et al.* (2007). The present study indicated that the period of pre-spermatogenesis was mitotically active. This was in agreement with that mentioned by Iczkowski *et al*. (1991). Contrastingly, Gondos *et al*. (1973) stated that, the period of pre-spermatogenesis was a quiescent period during which germ cell mitosis ceases followed by a second postnatally mitotic stage just before the onset of spermatogenesis. However, in rat, cessation of mitosis occurred between day 18 and day 19 in the fetus, and division did not resume until one week later, 4 days after birth, before spermatogenesis (Huckins and Clermont, 1968).

According to Gondos *et al*. (1973) and Iczkowski *et al*. (1991) who noticed increasing of mitosis just before the onset of spermatogenesis process, the current study revealed that the expression of DMRT1 was dramatically increased at 6w and reached peak at 6.5w which suggested that this increase might be due to sharp increase of mitosis just before the onset of spermatogenesis process. Noticeable, the increase of gene expression coincides with the appearance of late undifferentiated spermatogonia in seminiferous cord. This proves that DMRT1 was required to allow mitotic amplification before spermatogenesis and therefore, providing large numbers of spermatogonia that enter meiosis allowing abundant, continuous production of sperms. This coincides with that mentioned by Maston *et al*. (2010) and Don *et al.* (2011). The results presented here establish DMRT1 as a key regulator of the mitosis.

It is worth to mentioning that, spermatogenesis begins in New Zealand rabbits at 7-8week and when we investigated the level of mRNA pro-

duction for DMRT1 gene at this period; the expression of the gene was decreased. This proves that by onset of spermatogenesis, the expression of DMRT1 was decreased. As mentioned before, the expression decreased with the onset of spermatogonial differentiation (Matson *et al*., 2010). Dissimilar situation has been reported in the rainbow trout (Marchand *et al*., 2000), where DMRT1 expression was found to be highly expressed throughout spermatogenesis. Notably, at the level of histological analysis, the present study found that the late undifferentiated spermatogonia underwent maturation and transformed to spermatogonia at 7-8weeks postnatally. On the other hand, examination of expression of DMRT1 revealed decrease in expression at that stage. This result can be interpreted by that *DMRT1* was less abundantly expressed in differentiating spermatogonia as reported by Don *et al*.(2011)

Furthermore, the histological examination of 3 months-old rabbit testis revealed the appearance of primary spermatocytes in some seminiferous tubules and when following the gene expression, the expression was decreased than the previous age. This result can be interpreted by absence of DMRT1 expression in primary spermatocytes. This was in agreement with that mentioned by Maston *et al*. (2010) and Don *et al*. (2011) by in situ-hyperdization study.

RT-PCR data for DMRT1 of 4 months and 6 months rabbit testis revealed marked low expression near background. This decrease might be due to increased meiosis at these ages. This result was in agreement with that reported by Maston *et al*. (2010). Also, Marchand *et al*. (2000) mentioned that, the expression of DMRT1 decreased in rainbow trout at spermiation.

From histological view at these ages, all spermatogenic cells (spermatogonia, spermatocyte, spermatid and spermatozoa) appeared and when the gene expression was analyzed, marked decrease in the level of expression of *DMRT1* was found. This result can be interpreted by absence DMRT1 in premeiotic cells and postmeiotic cells (Maston *et al*., 2010 and Don *et al.,* 2011). These cells were the major cell type in seminiferous tubules at this age. Also, Johnsen *et al*. (2010) mentioned that in the adult males, abundant dmrt1 mRNA was restricted to the periphery of the tubuli, whereas no signal was identified in the more central region harbouring the spermatocytes and mature spermatids. Moreover, the present study examined the timing of STRA8 expression during postnatal testis development. STRA8 expression was detected in

testis soon after birth. However, its expression appeared to remain relatively stable from 0day to 4w and was present in low level comparing with the following ages which suggests the low expression of this gene in the presence of undifferentiating spermatogonia. This interval revealed the presence of undifferen tiating spermatogonia by the histogical analysis of testis.

The present study observed that the expression of STRA8 was releatively increased just before and during spermatogensis process which might suggest that STRA8 may promote spermatogonial differentiation. This result was consistant was that reported by Endo *et al*. (2015).

Interestingly, the histological analysis of rabbit testis at the age of (6-6.5ws) revealed the presence of late undifferentiated spermatogonia. At the same time, the expression level of STRA8 was releatively increased than the previous ages which might suggest that by the appearance of late undifferentiating spermatogonia, the expression increased. This result could be interpreted by that STRA8 expression also occurs in late undifferentiated spermatogonia in accordance with that reported by Endo *et al*. (2015). Also, Oulad-Abdel-ghani *et al*. (1996) mentioned that, STRA8 expression begins immediately before spermatogonial differentiation in mice. Thus, we could conclude that STRA8 expression begins in late undifferentiated spermatogonia.

At 7-7.5weeks age, spermatogenesis began at which the late undifferentiated spermatogonia underwent maturation and transformed to spermatogonia. At this stage, the expression level of this gene was increased than in the previous ages which might suggest that by the appearance of differentiated spermatogonia, the expression of gene increased. This result was consistant with Oulad-Abdelghani *et al*.(1996). This indicated that STRA8 might promote spermatogonial differentiation as reported by Endo *et al*. (2015).

From histogical view of rabbit testis at the age of 3months, primary spermatocytes were appeared. At the same time, the expression level of STRA8 was increased than in the previous ages which might suggest by the appearance of primary spermatocytes, the ex-pression increased. As already reported by Zhou *et al*. (2008); Endo *et al*. (2015) and Mark *et al*. (2015), STRA8 expression increased in premeiotic cells (primary spermatocyte). This pattern suggests that STRA8 is required for meiosis of the primary spermatocytes and STRA8 protein

may be play a role in the premeiotic phase of spermatogenesis.

Adult rabbit testes at age of 4- 6 months are in waves of meiosis and continue throughout life, providing a continuous supply of sperm from spermatogonial stem cells at the periphery of the testis cords and sperms appeared in the center of seminiferious tubules at this stage. In the current study, RT-PCR recorded high level of expression of STRA8 at this stage and the rabbit testis of 6 months where the volume of testis increased and germ cells which required more miosis increased. This study revealed that the expression of *STRA8* reached its peak which might suggest that STRA8 may play role in meiosis process. As already said, STRA8 is essential for successful meiosis and normal spermatogenesis (Zhou *et al*., 2008). Also, Daniel-carlier *et al*. (2013) mentioned that, in testes, the STRA8 gene was clearly expressed when meiosis started until adulthood.

This data displayed an interesting trend for STRA8 mRNA levels indicating low level of expression in the juvenile testis and rising sharply in a linear fashion to adulthood. This expression pattern would be in accordance with that STRA8 being required in the initiation of meiosis.

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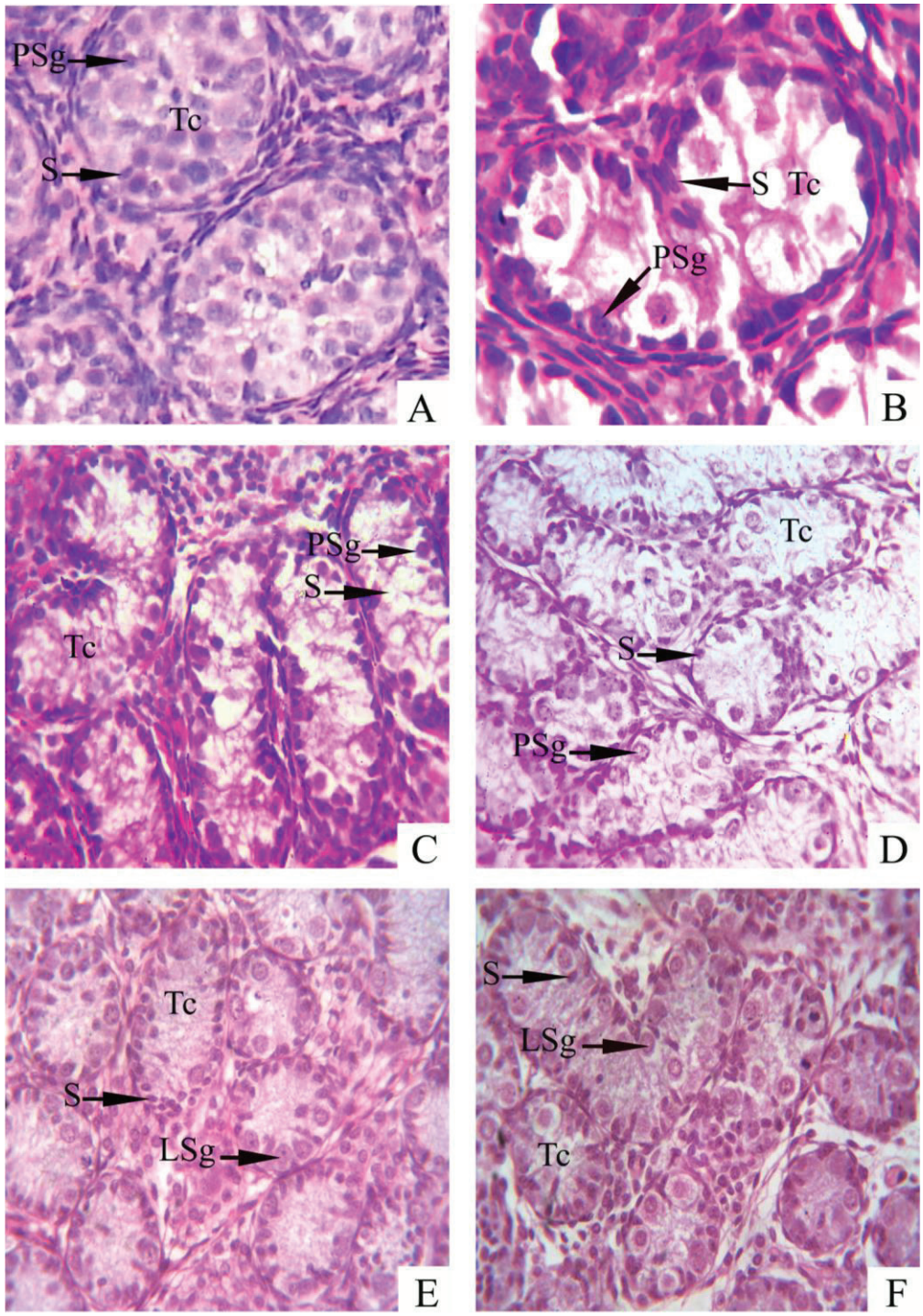
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Fig (1): (A, B & C): Photomicrographs of 0dpp, 2w and 4w old New Zealand rabbit testis respectively showing that: the testicular cords (Tc) contained two types of cell populations; small number of large, light, round cells with relatively round nuclei, prespermatogonia (PSg) and a large number of dark polygonal cells with irregular nuclei, Sertoli cells (S). **(D):** 6w and (**E)** 6.5w, the late undifferentiated spermatogonia appeared large with pale cytoplasm and large vesicular nucleus with clear nucleolus, (LPSg). H&E staining. X 40. Tc. testicular cord, PSg. Prespermatogonia, S. Sertoli cells, LPSg. Late undifferentiated spermatogoni

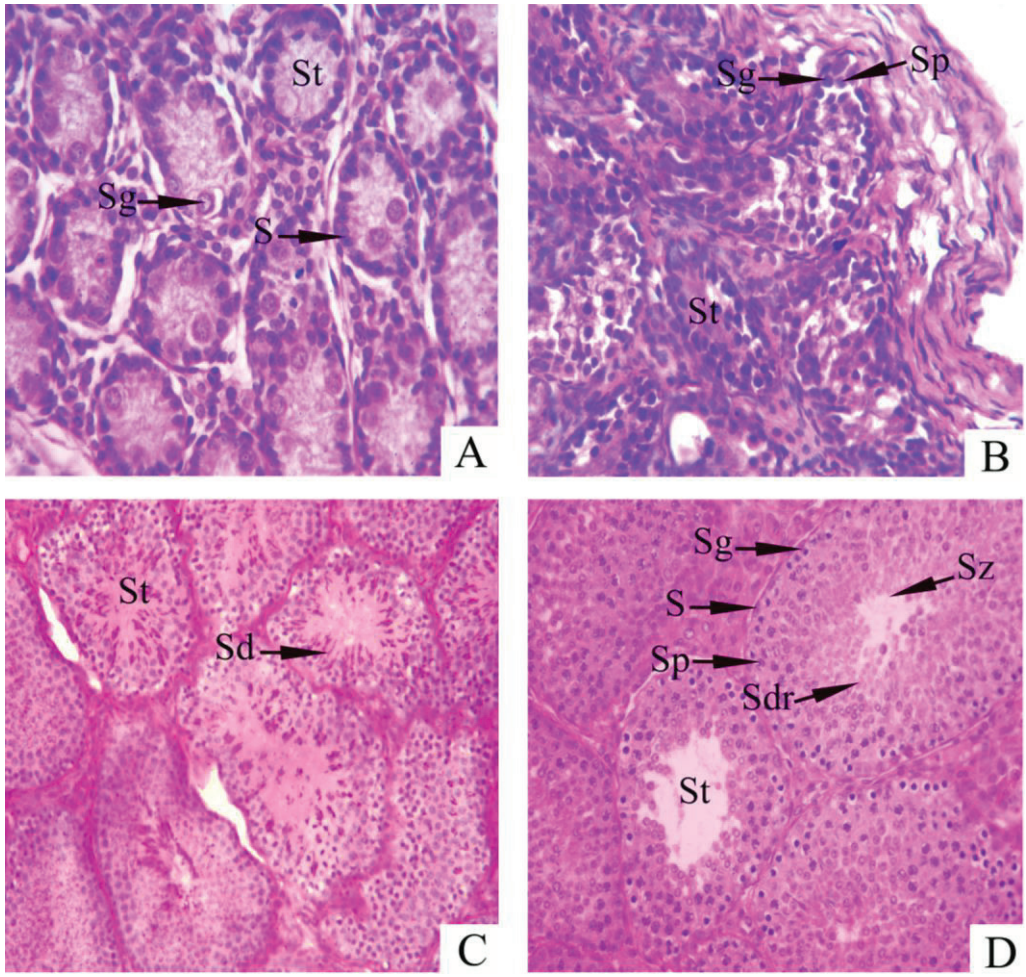

Fig (2): (A & B): Photomicrographs of 7w and 7.5 w old New Zealand rabbit testis respectively showing that: The spermatogenesis began at which the late undifferentiated spermatogonia (LSg) underwent maturation and transformed to spermatogonia (Sg) which appear spherical with clear cytoplasm and large round nuclei with condensed chromatin island peripherally seated and their nuclei were central or eccentric and vesicular. (B): 3m, the primary spermatocytes (Sp) observed in some seminiferous tubule. (C) 4m, Spermatids (Sd) were first seen at these stages. (D): 6m, all stages of spermatogenesis were evident and Spermatozoa (Sz) appeared in the tubular lumen at this stage. (A, B, D) H&E staining, (C) PAS staining. X 40. St seminiferous tubules, Sg. Spermatogonia, S. sertoli cells, Sp. primary spermatocyte, Sd spermatid, Sdr. round spermatid, Sz. Spermatozoa.

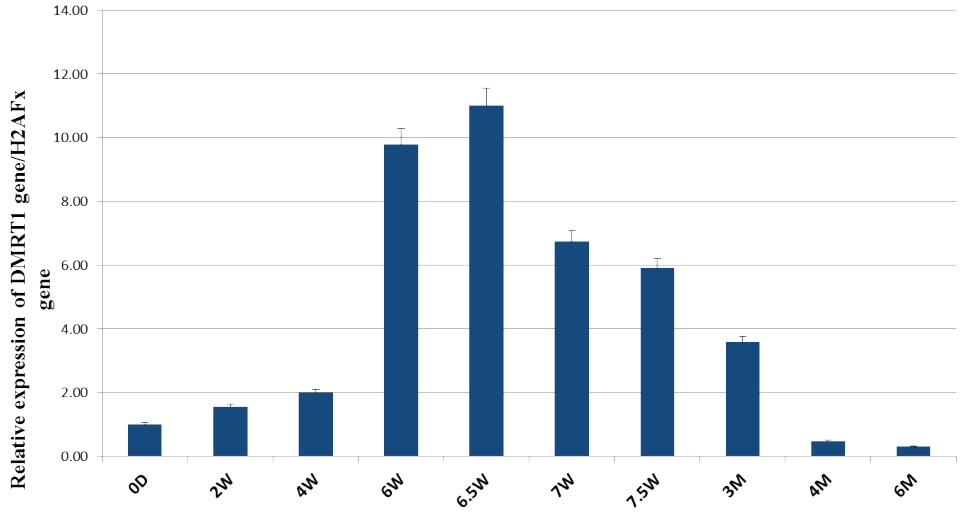

Fig (3): Graphical presentation of quantitative real-time PCR analysis of the expression of DMRT1 gene in rabbit testis from the age of 0dpp to 6M.

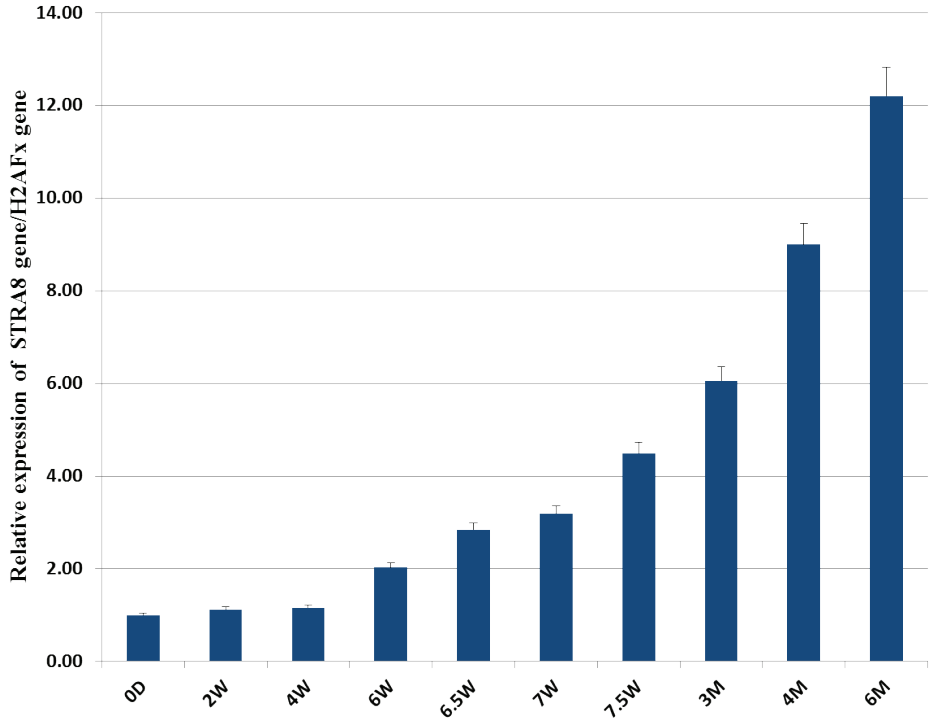

Fig (4): Graphical presentation of quantitative real-time PCR analysis of the expression of STRA8 gene in rabbit testis from the age of 0D to 6M.

Animals of this issue

Rabbit *(Oryctolagus cuniculus)*

Kingdom: Animalia & Phylum: Chordata & Class: Mammalia & Order: Lagomorpha & Family: Leporidae (in part) & Genus: *Oryctolagus*

The rabbit's long ears, which can be more than 10 cm (4 in) long, are probably an adaptation for detecting predators. They have large, powerful hind legs. The two front paws have 5 toes, the extra called the dewclaw. The hind feet have 4 toes. They are plantigrade animals while at rest; however, they move around on their toes while running, assuming a more digitigrade form. Wild rabbits do not differ much in their body proportions or stance, with full, egg-shaped bodies. Their size can range anywhere from 20 cm (8 in) in length and 0.4 kg in weight to 50 cm (20 in) and more than 2 kg. The fur is most commonly long and soft, with colors such as shades of brown, gray, and buff. The tail is a little plume of brownish fur (white on top for cottontails). Rabbits can see nearly 360 degrees, with a small blind spot at the bridge of the nose.

Source: Wikipedia, the free encyclopaedia

Morphometric Studies on the One-Humped Camel Foetus (C*amelus dromedarius*)

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Abstract

The relationships between the body dimensions, age and weight in the eighty-seven foetuses were determined. Each foetus was weighed and its body dimensions were measured using a string and a measuring tape. The sexes of the foetuses were determined by observing and recording the gonad type in the ventral caudal area. The age of each foetus was determined by measuring the crown-rump length. The mean (± SEM) weight of the foetuses ranged from 50.7 ± 7.4 g - 14350 ± 3372 g. The crown-rump length and body length of the foetuses varied from 15.8 ± 1.2 cm - 104 ± 8.5 cm and 20.5 ± 1.7 cm - 137 ± 11.7 cm, respectively. The tail length and neck length ranged from 3.3 ± 0.2 cm - 24.5 ± 2.4 cm and 5.7 ± 0.5 cm - 36.9 ± 2.6 cm, respectively. The relationships between the mean crown-rump length and body length of the foetuses were highly significant ($P < 0.001$). The mean crown-rump length of the foetuses were significant ($P < 0.001$) correlated with age, body weight, body length, tail length and neck length. Body length was significantly ($P < 0.001$) related to age, weight, tail length and neck length. The increase in tail length to neck length occurred at a fairly constant ratio of 1:8.

Keyworda: Morphometric, Camel (*Camelus dromedarius*), Kano abattoir.

Introduction

The camel (*Camelus dromedarius*) belongs to the family, *Camelidae.* This family is divided into three genera. The old-world camels-(i) genus *Camelus* the new world camel-(ii) genus *Lama* and (iii) genus *Vicugna* (Wilson and Reeder, 2005).

The mean gestation period of the camel is between 315-360 days (Puschmann, 1989) and May be up

to 370-375 days (Fazil and Hofmann, 1981; Arthur, 1992).

The dromedary camel (one-humped camel) is the most important animal to man in the semi-arid areas of Northern and Eastern Africa as well as in the deserts of the Arabian Peninsula. It is a multi-purpose animal, used for its supply of milk, meat, hides and for transport (Schwartz and Dioli, 1992; Farah, 2004; Kane *et al*., 2005; Kadim *et al*., 2008).

There is paucity of information on the developmental morphology of organs in the camels, including the lung

This study was conducted to determine the anatomical data of the camel foetus using readily available abattoir specimen.

Materials and Methods

Study area: The study was carried out in Kano State. It is located in the North-Western part of Nigeria and covers an area of 20,131 km^2. Kano State borders Katsina to the north-west, Jigawa State to the north-east, and Bauchi and Kaduna States to the south (Roger, 2009).

Experimental Animals: Eighty-seven foetuses were used for the study. They were collected immediately after their dam were slaughtered and the sex of each foetus was recorded.

Morphometric Studies: Foetuses were weighed to the nearest 0.1 g with weighing balance after being carefully dried with a clean towel. The following body dimensions were measured to the nearest 0.1 cm for each foetus using a string and a measuring tape.

Crowm-rump
length
Body weight
Body length of the foetus
Tail length
Neck length

Foetal Age Estimation: The gestational age of each foetus was estimated using a formula described by Elwishy *et al*., (1981) as follows: GA = (CRL + 23.99)/0.366. Where GA = Gestational age; CRL = Crown – rump length.

Data Analyses: All recorded values were expressed as means ± standard errors of mean. The statistical package used was Graphpad prism Software, San Diego, California, USA
(WWW.Graphpad.com)
WWW.Graphpad.co).Values of $P < 0.05$ were considered significant.

Results

The age varied from 108.7 ± 3.3 days to 349.3 ± 23.3 days. The body length ranged from 20.5 ± 1.7 cm to 137 ± 11.7 cm, the crown-rump length ranged from 15.8 ± 1.2 cm to

104 ± 8.5 cm while the weight of the foetuses ranged from 50.7 ± 7.4 g to 14350 ± 3372 g.

The tail length varied from 3.3 ± 0.2 cm to 24.5 ± 2.4 cm and the neck length ranged from 5.7 ± 0.5 to 36.9 ± 2.6 cm. (Table 1). In addition to the increase in age, body length and body weight, regular increase in tail length to neck length was observed at a fairly constant ratio of 1:8 (Table 3).

The crown-rump length and body length were significantly ($P < 0.001$) and directly correlated. The crown-rump length of the camel foetus was significantly ($P < 0.001$**)** and positively correlated with age, body length, body weight, tail length and neck length (Table 2).

Discussion

The mean crown-rump length of the camel foetus ranging between 15.8 ± 1.2 cm to 104 ± 8.5 cm obtained in this study is less than that of 17.3 ± 0.4 cm to 116.7 ± 1.8 cm, reported by Hussein *et a.,* (1991). The mean body length of the camel foetus, which ranged between 20.5 ± 1.7 cm to 137 ± 11.7 cm in the present study, is less than the range of 22.4 ± 0.5 cm to 154.7 ± 3.2 cm, reported by Hussein *et al.* (1991). The body weight of the camel foetus was 48.6 ± 4.0 g to 20933.3 ± 2547.1 g and the values were different ($P < 0.05$) from the mean body weight of camel foetus (50.7 ± 7.4 g to 14350 ± 3372 g) obtained in this study. The differences in the dimension obtained in the present study and that of Hussein *et al.* (1991) may be due to the variation in size of the animals, environmental condition and nutritional factors (Sivachelvan *et al*., 1996).

Body measurements steadily increased in the present study with increasing gestational age, and the crown-rump length was highly correlated ($P < 0.01$) with gestational age ($r = 0.9546$, $P < 0.001$).

Therefore, the developmental age may be estimated from foetus crown-rump length. In addition, since the correlation obtained between crown-rump length and body length was high, body length may also be used to estimate camel foetal age. This finding is in agreement with the findings of Elwishy *et al.* (1981) and Hussein *et al.* (1991) who obtained a highly significant correlation between gestational age and body measurements, and showed that the estimation of foetal age is possible with a fair degree of accuracy. Elwishy *et al.* (1981) suggested equations that were used to estimate the age of the camel foetus in days (X) from a known body dimension.

The result obtained by Hussein *et al.* (1991) in the one-humped camel showed that the ratio of radius to tibia

lengths indicates that the rate of the growth of the bones remains constant in relation to one another throughout the period of gestation. However, in this study, in addition to increase in the crown-rump length, body length, age and body weight, regular increase in tail length and neck length were observed at a fairly constant ratio of 1:8. The result obtained in the present study demonstrated, for the first time, the relationships between the morphometric parameters of the one-humped camel in the foetus, and that the parameters are directly related. The findings of the present study have demonstrated that the morphometric parameters of the foetus in the camel may be used to determine not only the size of the foetus, but also many morphometric anatomical parameters that are of value in the determination of the age of the foetus, crown-rump length, body length, body weight, tail length and neck length, using the regression equations obtained. Of particular clinical significance is the determination of body weight and age of the foetus using the regression equations generated from the present study. Besides the clinical value of the equations, the data obtained in the present study may be of value in forensic veterinary medicine, involving the determination of some unknown morphometric anatomical data of the foetus, especially the age in the camel.

Conclusion

Base-line anatomical data of the camel foetus on weight, body length, crown-rump length, tail length and neck length were obtained to the best of our knowledge in Nigeria.

Body weight of camel foetus and other body dimension studied were highly correlated.

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Table (1): Crown-rump, Body Weight, Body Length, Tail Length and Neck Length Length of the Camel Foetus (Mean ± SEM)

Crown-rump length Range	n	Crown-rump length (cm)	Age (Days)	Body length (cm)	Body weight (g)	Tail length (cm)	Neck length (cm)
10.5-19	7	15.8 ±1.2[a]	108.7 ± 3.3	20.5 ± 1.7	50.7 ± 7.4	3.3 ± 0.2	5.7 ± 0.5
20-25	9	23.1 ±0.4[a]	128.6 ± 1.1	30.9 ± 1.1	135.6 ± 13.3	5.1 ± 0.2	8.9 ± 0.4
26-37	29	33.6 ±0.7[b]	157.3 ± 1.9	43.3 ± 0.9	425.2 ± 29.1	7.0 ± 0.1	12.6 ± 0.4
38-44	14	42.0 ±0.6[c]	251.6±71.4	54.2 ± 1.0	766.1 ± 50.2	9.0 ± 0.3	16.1 ± 0.3
45-54	12	49.0 ±0.9[d]	199.2 ± 2.5	64.0 ± 1.2	1427 ± 123.9	10.3 ± 0.3	18.9 ± 0.5
55-65	5	61.9 ±1.1[a]	234.4 ± 3.0	81.6 ± 1.7	2590 ± 202.1	13.5 ± 0.7	24.3 ± 1.3
66-80	5	71.1 ±2.2[a]	259.6 ± 5.7	93.2 ± 3.0	4360 ± 567.3	16.4 ± 1.1	27.7 ± 1.0
81-90	3	83.2± 1.4[e]	293.6 ± 3.6	109.7 ± 2.0	7283 ± 683.9	20.5 ± 0.9	33.1 ± 1.0
91-125	3	104 ± 8.5[f]	349.3±23.3	137 ± 11.7	14350 ± 3372	24.5 ± 2.4	36.9 ± 2.6

[a,b,c,d,e] and [f]= Values with different superscript letters within the same column are statistically ($P < 0.05$) significant.
n = Number of camel foetuses sampled

Table (2): Relationships between Age and Body Dimension of the Foetus of the one-humped Camel (*Camelus dromedarius*) (n = 87)

Correlated parameters	Correlation coefficients
Crown-rump length and age	0.9546***
Crown-rump length and body length	0.9999***
Crown-rump length and body weight	0.9170***
Crown-rump length and tail length	0.9968***
Crown-rump length and neck length	0.9948***
Body length and age	0.9523***
Body length and weight	0.9186***
Body length and tail length	0.9971***
Body length and neck length	0.9947***
Body length and crown-rump length	0.9999***

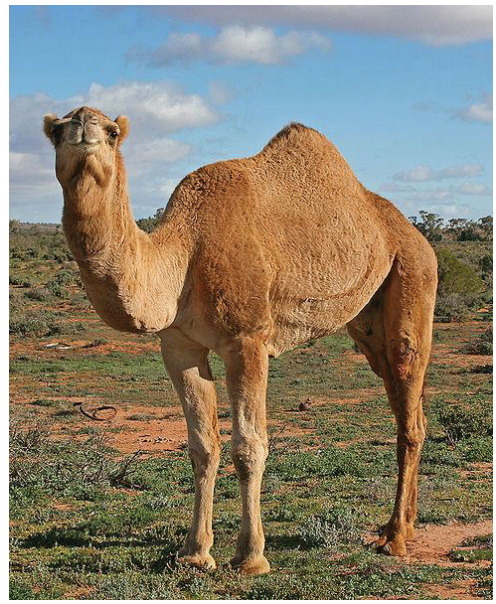
*** = $P < 0.001$

Table (3): Length of the Neck and Tail and the Neck:Tail Ratio during the Developmental Stages of One-humped Camel (*Camelus dromedarius)* Foetus.

		Average length		
Range of Crown-rump Length (cm)	N	Neck (cm)	Tail (cm)	Neck: Tail Ratio
10.5 – 19	7	5.7 ± 0.5	3.3 ± 0.2	1.7
20 – 25	9	5.1 ± 0.2	8.9 ± 0.4	1.7
26 – 37	29	7.0 ± 0.1	12.6 ± 0.4	1.8
38 – 44	14	9.0 ± 0.3	16.1 ± 0.3	1.8
45 – 54	12	10.3 ± 0.3	18.9 ± 0.5	1.8
55 – 65	5	13.5 ± 0.7	24.3 ± 1.3	1.8
66 – 80	5	16.4 ± 1.1	27.7 ± 1.0	1.7
81 – 90	3	20.5 ± 0.9	33.1 ± 1.0	1.6
91 – 125	3	24.5 ± 2.4	36.9 ± 2.6	1.5

Animal species in this issue

One-humped came (*Camelus dromedaries*)



Kingdom: Animalia, Phylum: Chordata, Class: Mammalia, Oder: Artiodactyla. Family:Camelidae, Genus: *Camelus*

Camel is an even-toed ungulate within the genus ***Camelus***, bearing distinctive fatty deposits known as humps on its back. There are two species of camels: the dromedary or Arabian camel has a single hump, and the Bactrian camel has two humps. They are native to the dry desert areas of West Asia, and Central and East Asia, respectively. Both species are domesticated to provide milk and meat, and as beasts of burden.

The average life expectancy of a camel is 40 to 50 years. A fully grown adult camel stands 1.85 m at the shoulder and 2.15 m at the hump. The hump rises about 30 inches (76.20 cm) out of its body. Camels can run at up to 65 km/h (40 mph) in short bursts and sustain speeds of up to 40 km/h (25 mph).

Fossil evidence indicates that the ancestors of modern camels evolved in North America during the Palaeogene period, and later spread to most parts of Asia. Humans first domesticated camels before 2000 BC.

Camels are able to withstand changes in body temperature and water content that would kill most other animals. Their temperature ranges from 34 °C at night and up to 41 °C during the day, and only above this threshold will they begin to sweat.

Determination of the Proportions of Muscle Fibre Types from Selected Muscles of the Forelimb: A Comparative Study of Cattle (*Bos taurus indicus*) and One-humped Camel (*Camelus dromedaries*)

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Abstract

A total number of fifty forelimbs comprising Twenty-five forelimbs male dromedary camels (*camelus dromedaries*) and those of male cattle, Zebu type, (*Bos taurus indicus*) within the ages of 6 months, 1 year, 3 years, 5 years and 7 years, were purchased from Sokoto Municipal Modern abattoir. Selected muscles sampled (1 cm^2) from the forelimbs of both cattle and camel were taken from the middle part of the muscle bellies (from the biceps brachii, triceps brachii and deltoideus), fixed in Bouin's solution and prepared for histochemical analyses.

The photomicrographs obtained from the biceps brachii, deltoideus and triceps brachii among both cattle and camel demonstrated the morphology of the different fibre types (type I, type IIA and type IIB). Muscle fibre types showed different proportions. Even though the overall mean proportion of muscle fibre type I, type IIA and type IIB could not present any significant ($p>0.05$) differences between the camel and the cattle looking at it holistically. As revealed in this work, the effects of the interactions of species versus age, species versus muscle and muscle versus age on the proportion of muscle fibre type I revealed that the deltoideus muscle of 1 year old camel had significantly ($p<0.05$) higher proportion. Type IIA muscle fibre obtained from the deltoideus muscle of 5-year-old camel showed a signifi-

cantly highest value, while muscle fibre type IIB showed significantly higher value from the triceps brachii of cattle aged 1 year. The importance of the knowledge of microscopic characteristics in the determination of myofibre types could be of help in advancing the knowledge on muscle morphology (anatomy) and as well be of help in meat science industry.

Keywords: Muscle Fibre, Proportion, Triceps Brachii, Biceps Brachii, Deltoideus, Cattle; Camel.

Introduction

The phenotypic differences among skeletal muscle cells, termed fiber types, their potential for adaptation and underlying mechanisms have been a topic of study for several decades. In mammals, skeletal muscles are composed of a mixed population of red, intermediate and white muscle fibres (Close, 1972). Most mammalian skeletal muscles are composed of varying proportions of the three major types (I, IIA and IIB) of fibers, while certain muscles such as the rat soleus consist predominantly of type I fibers (Dimov and Dimov, 2007).

Skeletal muscles are composed mainly of three myofibre types that differ in histochemical properties, which reflect physiological and functional aspects of myofibres (Suzuki *et al*., 1999). Myofibres are classified into types I, IIA, and IIB by differences in reactivity for myosin ATPase and dehydrogenases. Type I myofibres correspond to slow-twitch/oxidative myofibres, type IIA myofibres to fast-twitch/ oxidative/glycolytic myofibres, and type IIB to fast-twitch / glycolytic myofibres (Handel and Stickland, 1987). The distinctive physiological properties of different skeletal muscle fibre types allow the muscle to respond to various mechanical (speed and endurance) and metabolic (anaerobic or aerobic) demands (Pette, 2001).

Paucity of information particularly on the muscle histochemical profile in camel and cattle is a driving force in this present research, considering the utility of these animal species in different fields. Similarly, observation of the proportion of the muscle fibre types in these animal species may help at elucidating general principles of musculoskeletal function and design, which could find application in Anatomy and Meat Science Industries.

Materials and Methods

Twenty-five forelimbs of male one-humped camels (*Camelus dromedaries*), and twenty-five of male cattle ,Zebu type, (*Bos taurus indicus*), all within the ages of 6 months, 1, 3, 5 and 7 years, were purchased from

Sokoto Municipal Modern abattoir. The age of each animal was determined using the method of Wilson (1984) and Dyce *et al.* (2010) to determine their ages, while evaluation of the animals to exclude any animal with musculoskeletal deformity or diseases was done through physical examination. The live body weights of the animals were estimated using linear body measurement based on the formula used by Yagil, (1994).

The samples (forelimbs) obtained were wrapped in clean sterile polyethylene bags and transported in a clean cool box containing ice cubes to the laboratory of the Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto-Nigeria, where the triceps brachii, biceps brachii and the deltoideus muscles were all carefully dissected out using the methods of Chibuzo (2006) as slighly modified by Sonfada (2008), noting the origin and insertion of every muscle before further different processing and analyses followed.

After the dissection and isolation of the muscles of interest, scalpel blade was used to excise portions of muscle samples measuring about 1 cm^2 (measurements were obtained with the aid of a tape rule, placed across the muscle section) from the forelimbs of both cattle and camel were taken from the middle part of the muscle bellies (from the biceps brachii, triceps brachii and deltoideus), fixed in Bouin's solution and prepared for histochemical analyses using the method of Dubowitz (1985) as a guide. This procedure helped in demonstrating the specific muscle fibre types from the selected muscles. After histological preparation of the slides, the prepared slides were viewed using a microscope (Olympus® CH 23, Germany) at different magnifications (x40, x100, x400). Photomicrographs were then obtained and transferred into a computer (Compac® Laptop, HDM, Presario CQ60) for further evaluation and detailed studies. The area and number of muscle fibres were calculated from 5 randomly selected fields. The proportions of muscle fibre types were calculated by dividing the number of each muscle fibre types by the total number of muscle fibre types as indicated by Fuentes *et al*. (1998).

Numerical data obtained were reported as mean±SD (Standard deviation) and presented in form of tables and charts. Data generated from the study were analyzed following a completely randomized design with a factorial arrangement of treatments using the general linear model (GLM) of SPSS (Version 16.0, 2007). Statistical significance of experimental observations was set at $P<0.01$ and $P<0.05$ where appropriate. All statistical analyses were done using SPSS (Version 16.0, 2007).

Results

The photomicrographs obtained from the biceps brachii, deltoideus and triceps brachii from both cattle and camel demonstrated the morphology of the different fibre types (type I, type IIA and type IIB) as presented in Plates (1) and (2).

Even though the overall mean proportion of muscle fibre type I, type IIA and type IIB could not present any significant ($p>0.05$) differences between the camel and the cattle looking at it holistically (Table 2). As revealed in this work, the effects of the interactions of species versus age, species versus muscle and muscle versus age on the proportion of muscle fibre type I revealed that the deltoideus muscle of 1 year old camel had significantly ($p<0.05$) higher proportion (Table 1, Figures 1, 2 and 3). Type IIA muscle fibre obtained from the deltoideus muscle of 5-year-old camel showed a significantly highest value (Table 1; Figure 4), while muscle fibre type IIB showed significantly higher value from the triceps brachii of cattle aged 1 year (Table 1; Figures 5 and 6).

Discussion

Although the overall mean proportion of muscle fibre types I, IIA and IIB could not present any significant ($p>0.05$) differences between the camel and the cattle in this work, the effects of the interactions of species versus age, species versus muscle and muscle versus age on the proportion of muscle fibre type I revealed that the deltoideus muscle of 1 year old camel had significantly ($p<0.05$) higher proportion. The observed larger proportion of muscle fibre type I in triceps brachii irrespective of the species, is indicative that this muscle is larger and heavier than other muscle types. This observation is consistent with the report of Fuentes *et al*. (1998) that who furthermore also observed that type I fibre is designed for slow body movements and are significantly present in postural muscles. *Muscle fiber types differ between individuals, but they also differ between muscles. Some muscle groups are very slow twitched (they have a high type I muscle fiber proportion) and other muscle groups are very fast twitched (they have a high type II muscle fiber proportion), although most muscle groups display an even mixture of both fiber types.* The musculature of the forelimbs in larger animals appears to be more involved with maintaining a posture and as well aiding in propulsive movements, even though in this present research the individual roles of the limbs/muscles were not assessed.

All effects of interactions on type II A muscle fibre proportions indicated that the deltoideus muscle of 5 years

old camel, triceps brachii of 3 years old camel and deltoideus of 3 years old cattle all had higher significant ($p<0.05$) values. All interactions on the proportion of muscle fibre type IIB indicated that 1 year old camel and cattle both had higher proportions ($p<0.05$), with the biceps brachii of camel having the highest value which was closely followed by the triceps brachii. This is consistent with the findings of Sonfada (2008) in camel, who reported that biceps brachii muscle showed the presence of fast twitch (white fibres) predominating the red fibres. That is possibly why camels can walk for long distance with sustained activitity and sometimes even being used in racing. In the same vein, it has been reported by Gonyea *et al*. (1981) that the proportion and regional distribution of muscle fibre types within a muscle appear to be related to the degree of functional complexity.

In agreement to the findings of Kadim *et al*. (2009) on their work on camel and cattle, and to the work of Brandstetter *et al.* (1998) on fibre characteristics in growing bull, this present work indicated that muscle fibre type IIB had the highest mean proportion followed by muscle fibre type IIA and type I from both the camel and cattle studied. For both species studied in this work, higher proportion of type IIB fibres is in agreement with Wegner *et al.* (2000), although with different distribution pattern for the other fibres types (types I and IIA).

The fact that muscle fibre type IIB (white fibres) predominate in the biceps brachii and triceps brachii muscles may be due to the fact that these muscles are under constant tension due to their structural, supportive and propulsive roles in these animal species thus depleting them of their glycogen content. Another possible reason could be due to the likely conversion of muscle fibres between types IIA and IIB as was reported by Brandstetter *et al.* (1998), with emphasis being laid on the preference of type IIB fibres even at an early stage after birth (Picard, *et al.*, 1995). Kassem *et al.* (2004) also reported that in camel muscles, type IIA fibres had the highest percentage followed by type IIB and type I respectively. The differences between the finding of the current study and that of Kassem *et al.* (2004) might be attributed to variations between the camels due to the heterogeneity of dromedary camels, as there are no established pure camel breeds as obtainable in other species, and this could also be probably due to differences in muscle type as they used longissimus thoracis muscle in their study.

A myofibre's indigenous physiological and metabolic properties contribute to the effectiveness and efficiency of the functioning of the skeletal muscle with respect to the support and movement of the body. The most comprehensive system of nomenclature for myofibre 'typing' gives a full description of these physiological and metabolic capacities, based on the relative contraction speed of the fibre and its propensity for oxidative and glycolytic metabolism (Handel and Stickland, 1987).

In general, the proportion of muscle fibre is related to changes throughout growth, and fast-growing farm animals were known to have more muscle fibres than slower growing ones. Within strain, the fibre number may increase with increasing average daily gain and gain: feed ratio (Stickland, 1995).

The importance of the knowledge of microscopic characteristics in the determination of myofibre types has been stated by several works, especially the parameters concerning meat quality (Lawrie, 1974). According to this author, meat quality is largely influenced by the changes in the morphology and muscle fibre composition during growth. The adoption of morphological and histochemical methods was also useful in the study of changes in myofibre size, distribution, frequency and connective tissue of different breeds of lambs (White *et al*., 1978; Sivachelvan and Davies, 1986; Suzuki and Tamate, 1988). Thus, this present work could be of help in advancing the knowledge on muscle morphology and as well be of help in meat science industry.

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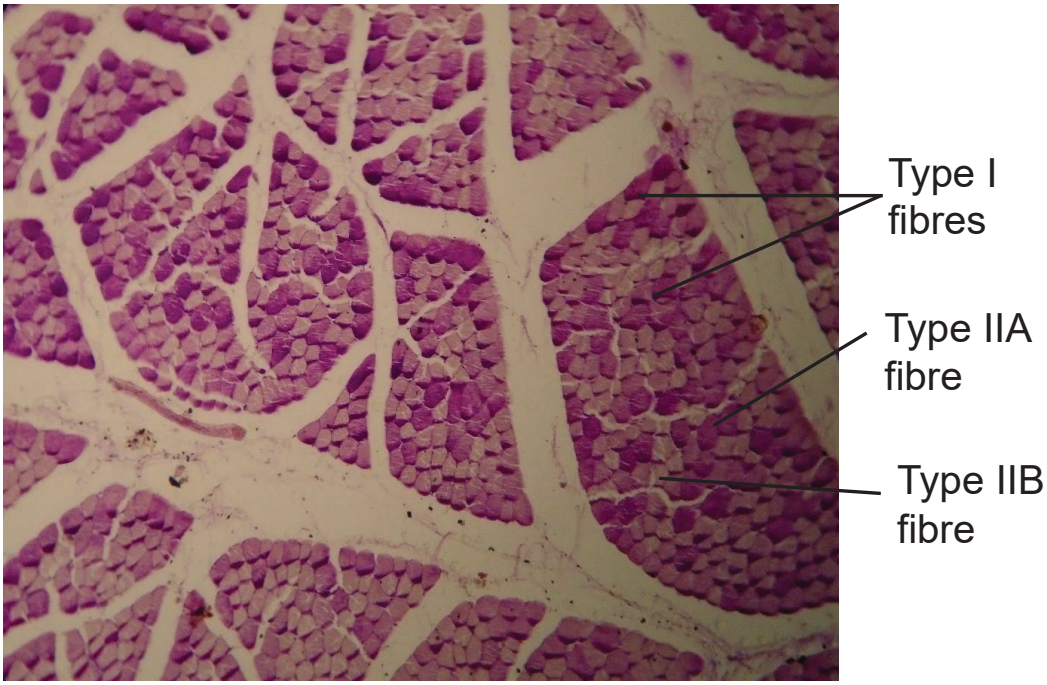

Plate (1): Photomicrograph of camel's deltodeus muscle demonstrating the fibre types (PAS x100).

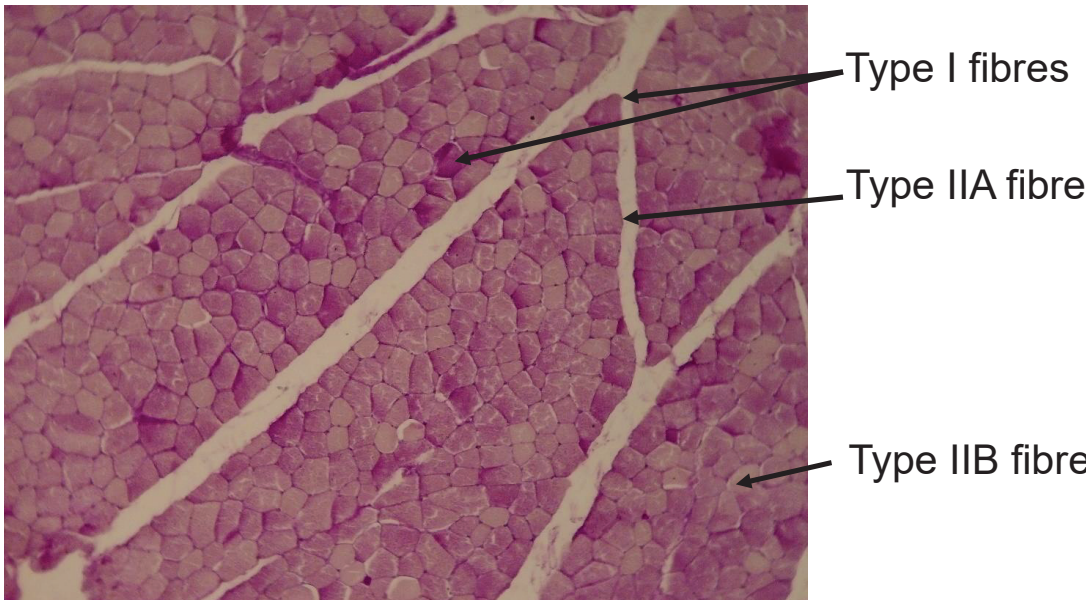

Plate (2): Photomicrograph of cattle's deltoideus muscle demonstrating the different fibre types (PAS x100)

Table (1): Proportions of Muscle fibre types in camel and cattle

Factors	Camel	Cattle	Camel	Cattle	Camel	Cattle
	Type I	Type I	Type IIA	Type IIA	Type IIB	Type IIB
6 MO						
BB	17±1.30[b]	32.2±1.59[a]	32.2±2.76[a]	17.6±1.21[b]	48.8±1.36	49.2±1.56
D	40.6±1.20[a]	34.2±1.77[b]	30±1.00	25±2.17	29.4±1.57[b]	40.8±2.56[a]
TB	18.2±1.46[b]	39.6±3.09[a]	30.2±2.82	37.2±2.22	51.6±3.44[a]	23.2±2.81[b]
1 YO						
BB	20.2±3.14[b]	35.6±2.16[a]	22.2±1.98[a]	14.4±1.86[b]	54.6±3.54[a]	50±2.70[b]
D	53.4±5.46[a]	36.8±1.56[b]	23.8±2.48[b]	29.8±0.86[a]	22.8±3.48[b]	33.4±1.50[a]
TB	52.3±2.08[a]	29.8±5.05[b]	14.8±1.43	15.6±2.20	32±1.58[b]	56.6±2.36[a]
3 YO						
BB	31±1.22[a]	20.2±2.59[b]	21±2.00	27.8±2.42	48±1.67	50.4±3.09
D	33.4±3.85	35±3.19	35±4.59	39.4±3.07	31.6±3.91	25.6±3.53
TB	33.6±3.88	32.8±2.59	38.2±3.56	36±2.41	27.8±6.77	31.2±2.82
5 YO						
BB	33±2.12	26.2±2.35	30.2±1.16	30.4±0.51	36.8±3.04	43.4±1.96
D	24.6±4.90	33.4±2.50	40.6±3.19[a]	27.6±4.94[b]	34.8±5.23	39±5.51
TB	50.6±2.58	46.2±3.97	17±1.52[b]	29.8±2.67[a]	32.4±1.89	24±4.37
7 YO						
BB	29.4±3.41	23.6±3.34	22±1.58[b]	29.4±2.16[a]	48.2±3.51	47±3.88
D	24.6±4.13	38±4.70	36.2±4.69	31.6±3.81	37.4±3.29	30.4±5.39
TB	44.8±5.58	44.2±5.23	28.6±3.83	31.8±5.39	26.6±5.33	24±3.78

Key: *MO = Months Old, YO = Year Old, BB = Biceps brachii, D = Deltoideus, TB = Triceps brachii*

[ab] Means bearing different superscript in the same row within a subclass differ ($p<0.05$)

Table 2: Overall mean proportions of muscle fibre types and their interactions in camel and cattle muscles

Factor	Percentages of muscle fibre types		
	Muscle fibre type I (%)	**Muscle fibre type IIA (%)**	**Muscle fibre type IIB (%)**
Species			
Camel	33.96 [a]	28.13 [a]	37.59 [a]
Cattle	33.92 [a]	28.23 [a]	37.88 [a]
SEM	1.07	0.82	1.07
Age			
6 months	30.47 [c]	28.70 [b]	40.83 [a]
1Year	38.17 [a]	20.10 [c]	41.40 [a]
3 Years	31.00 [bc]	32.90 [a]	35.77 [b]
5 Years	35.67 [ab]	29.27 [ab]	35.07 [b]
7 Years	34.40 [abc]	29.93 [ab]	35.60 [b]
SEM	1.69	1.29	1.67
Muscle			
Biceps	26.94 [c]	24.72 [c]	47.74 [a]
Deltoid	35.58 [b]	31.90 [a]	32.72 [b]
Triceps	39.30 [a]	27.92 [b]	32.74 [b]
SEM	1.31	1.00	1.31
Interactions			
S x A	**	NS	NS
S x M	*	NS	*
A x M	**	NS	**

[abcd] Means bearing different superscripts along the same column within a subclass differ ($p<0.05$)

Key: S = Species; M = Muscle; A = Age; SEM = Standard Error of the mean, NS = Not Significant; * $p<0.05$; ** $p<0.01$

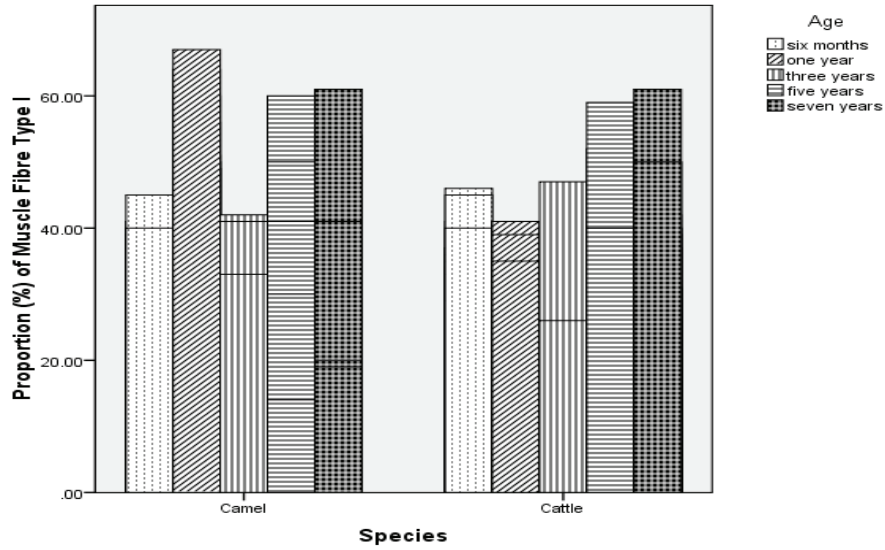

Fig (1): Effect of age versus species interaction on proportion of muscle fibre type I

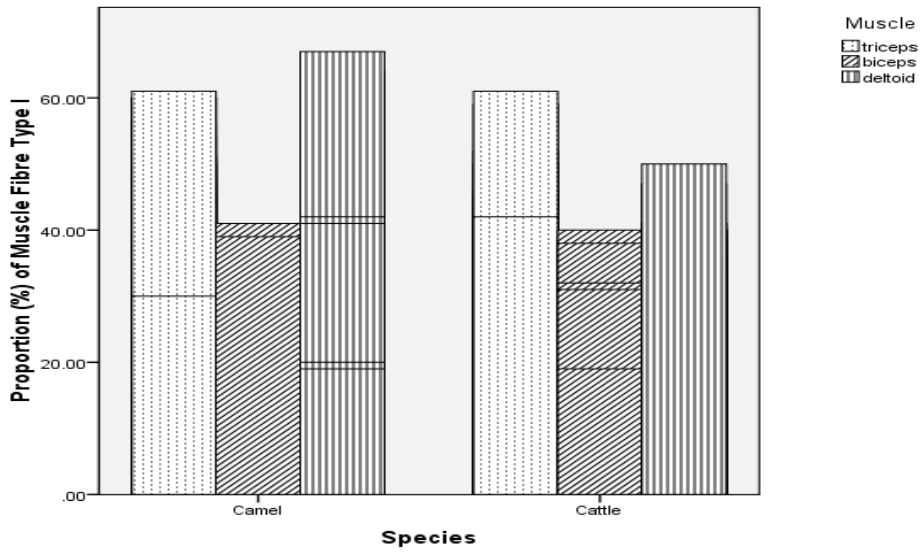

Fig (2): Effect of muscle versus species interaction on proportion of muscle fibre type I

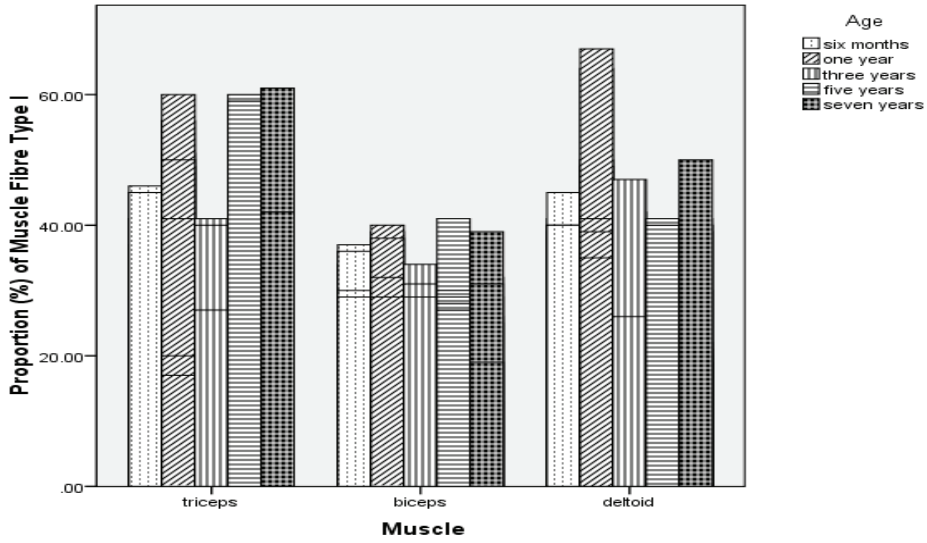

Fig (3): Effect of age versus muscle interaction on proportion of muscle fibre type I

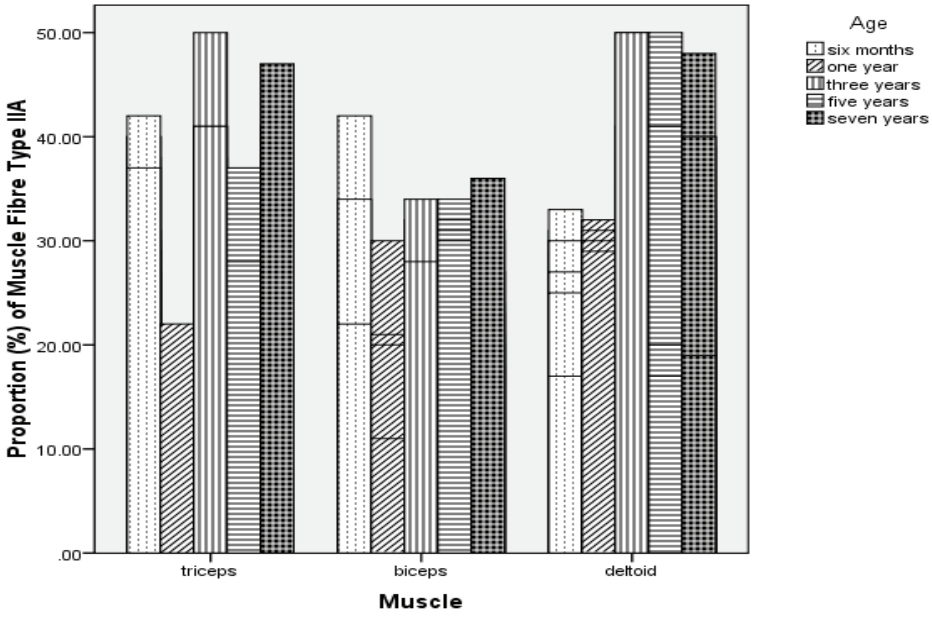

Fig (4): Effect of age versus muscle interaction on proportion of muscle fibre type IIA

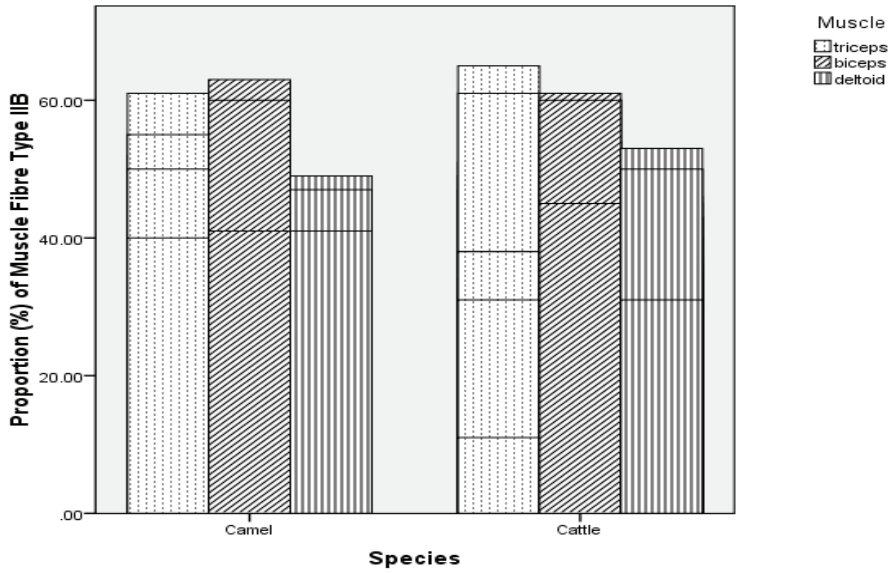

Fig (5): Effect of muscle versus species interaction on proportion of muscle fibre type IIB

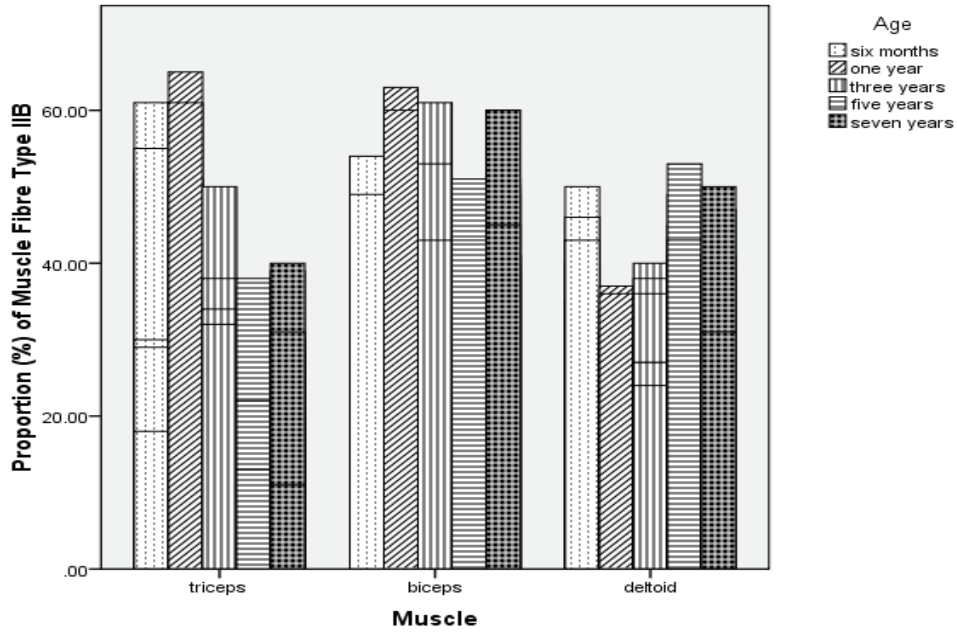

Fig (6): Effect of age versus muscle interaction on proportion of muscle fibre type IIB

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