Scholars Journal of Agriculture and Veterinary Sciences (SJAVS) **e-ISSN 2348–1854**
Abbreviated Key Title: Sch. J. Agric. Vet. Sci. **p-ISSN 2348–8883**

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Histological and Histochemical Studies on the Lacrimal Gland of Buffaloes (*Bubalus bubalis*)

Ibrahim Alhaji Girgiri, Pawan Kumar*
Department of Veterinary Anatomy, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004 (Haryana), India

Original Research Article

***Corresponding author**
Pawan Kumar

Article History
Received: 18.05.2018
Accepted: 28.05.2018
Published: 30.05.2018

DOI:
10.36347/sjavs.2018.v05i05.004

Abstract: The present study was conducted on 8 eyes of adult buffaloes. The lacrimal glands were collected and processed for histology and histochemistry. The gland was tubulo-acinar type and was surrounded by a thick capsule from which the connective tissue septae penetrated the parenchyma dividing the glandular tissue into lobes and lobules. The glandular acini had pyramidal shaped cells possessing strongly eosinophilic cytoplasm however; a few had lesser eosinophilic cytoplasm. The strongly basophilic round to oval nuclei were mainly pushed towards the base. These acini were predominantly mucous in nature and revealed presence of varying concentrations of different mucopolysaccharides. The acini were surrounded by myoepithelial cells. Plasma cells were generally present in small clusters in between the acini.
Key words: Buffaloes, Lacrimal gland, Histology, Histochemistry.

INTRODUCTION

The lacrimal gland (LG) is a capsulated organ, situated at the dorsolateral quadrant of the eye orbit [1]. It is a compound tubulo-acinar or tubulo-alveolar gland [2], with serous secretions in cats, and sero-mucous in dogs and ungulates [3]. The LG is mainly responsible for the production of aqueous portion of tear film responsible for moistening, nourishing and lubricating the anterior surface of the eye [4]. It also functions in defense system of the ocular surface as part of the conjunctiva-associated lymphoid tissue [5]. The histomorphology of the LG has been described in dog [6], small ruminants [7, 8], cattle and bison [9], and the camel [10, 11]. However, in the buffalo, such studies are scantly found in the available literature. The present study aims to report investigations on the structure of LG of buffaloes by light microscopic approach.

MATERIALS AND METHODS

The lacrimal gland samples used in the study were carefully excised from the eyeball structures of 4 heads of adult buffaloes (local mixed breed) of either sex obtained immediately after slaughter. The glands were fixed in 10% neutral buffered formalin for 48 hours and subjected to routine paraffin embedding for light microscopy. The paraffin sections of 5-6 µ were cut and stained with routine Harris' hematoxylin and eosin stain for general histological examination. The connective tissue fibers were demonstrated by Gomori's method for reticulum, Weigert's method for elastic fibers [12] and Crossman's trichrome stain for collagen fibers [13]. For histochemical studies, McManus' method for glycogen (PAS), PAS-Alcian blue method for acidic and neutral mucosubstances (pH 2.5), Alcian blue method (pH 2.5) for mucosubstances, Meyer's mucicarmine method for mucin, colloidal iron method for acid mucopolysaccharides [12] were employed. Fontana-Masson method was used for demonstration of endocrine cells [14].

RESULTS AND DISCUSSION

The lacrimal gland in the present study was compound tubulo-acinar type (Fig-1) as earlier reported in the cattle and bisson [9], roe deer [15], alpaca [16] and small ruminants [8]. Whereas; it was compound tubulo-alveolar in the camel [11], human [17] and dog [6]. The gland was enclosed by a thin capsule (Fig-1) having varying concentration of reticular, collagen and elastic fibers. The connective tissue septae from the capsule penetrated the parenchyma of the gland and divided it into lobes and lobules of unequal size. These connective tissue septae were mainly having the reticular fibers followed by few collagen and elastic fibers consistent with findings in the alpaca [16] and small ruminants [8]. However, the capsule was composed chiefly of collagen fibers in the Philippine water buffalo [18] and dog [19]. In the present study, the concentration of the collagen and reticular fibers was drastically increased at the interlobular area (Fig 2, 3 & 4) and in addition a few elastic fibers were also observed. There was mixed distribution of the acini

which were purely serous or mucous or mixed type as reported in the sheep [7] and dog [6, 19]. The serous type of acini was having pyramidal shaped cells and their nuclei were round to oval in shape, present towards the basement membrane (Fig 5 & 6) as reported in the sheep [7]. Their basophilic nuclei presented smaller clumps of chromatin especially towards the outer nuclear membrane and thus, presented vacuolated areas towards the center. The nucleolus was generally centric in position. The cytoplasm of these cells was finely granular and eosinophilic especially towards the supranuclear portion (Fig-6). The maximum eosinophilia was observed towards the luminal surface. The mucous acini were also lined by the pyramidal cells and their nuclei were elongated rod shaped and were pushed close to the basement membrane. These nuclei were strongly basophilic in nature because of uniform distribution of deeply basophilic chromatin material which also masked the appearance of the nucleoli as reported in roe deer [15]. The cytoplasm of the cells was finely granular and very lightly eosinophilic and at places presented floccular or vacuolated appearance because of the washing of the mucous during processing of the tissues. The intra and inter glandular ducts were lined by simple to stratified cuboidal type of epithelia as reported in the roe deer [15], small ruminants [8] and dog [19]. However, the interlobular ducts were lined by pseudostratified epithelium in the Iranian river buffalo [20], cattle and bison [9]. In the Philippine buffaloes, the interlobular ducts were lined by tall simple to stratified columnar epithelium [18].

The sero-mucous acini were surrounded by myoepithelial cells which had flat cells having densely or darkly basophilic elongated flattened nuclei as frequently observed in different species [18, 15, 6, 8]. The nuclei of these cells occupied majority of the portion of the cell and very thin eosinophilic cytoplasm was visible. The myoepithelial cells possessed characteristics of both muscle and epithelial cells and when stimulated plays an important role in propulsion of secretion [3]. Myoepithelial cells in the lacrimal gland (LG) as well as other exocrine glands are believed to synthesize the basement membrane and form a functional network around the acinar and ductal cells, separating them from the basement membrane and the mesenchymal stromal cell [21]. Helen and Darlene [22] mentioned that myoepithelial cells maintained glandular structural integrity and transport metabolites to secretory cells.

In addition to these serous and mucous type of cells, the present study also demonstrated a few comparatively large sized nuclei, some of which were lightly stained and others were darkly stained, and these nuclei were pushed towards the base of the acini. In between the clusters of these acini, large number of plasma cells, a few lymphocytes and fine blood capillaries were also observed (Fig-5 & 6). Similarly, clusters of lymphocytes in the form of small aggregates were observed especially towards the interlobular regions (Fig 1, 5). These cells were usual constituents of the lacrimal glandular interstitium [23, 24]. The lymphoid cells present in LG were constituent part of the lacrimal drainage associated lymphoid tissue which provided immunity at mucosal and glandular level [5, 25] and thus their ability to secrete immunoglobulins along with lacrimal fluid, conjunctival epithelium, lymphocytic redistribution and neural reflex mechanisms are strategic in protecting the mucosal surface [25]. The LG interstitial plasma cells produced secretory immunoglobulin A [26] protecting the sensitive mucosa and the ocular surface of the eye against viral infections, bacterial attachment and colonization, and parasite infestation [27]. The secretion of IgA could be regulated by hormones, immune factors and neural responses [28].

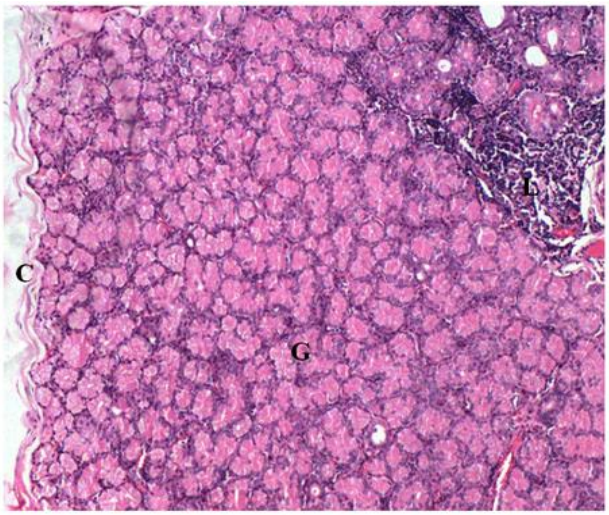

Fig-1: Photomicrograph of lacrimal gland of buffalo showing connective tissue capsule (C) glandular parenchyma (G) and lymphoida aggregation (L) H and E x 40

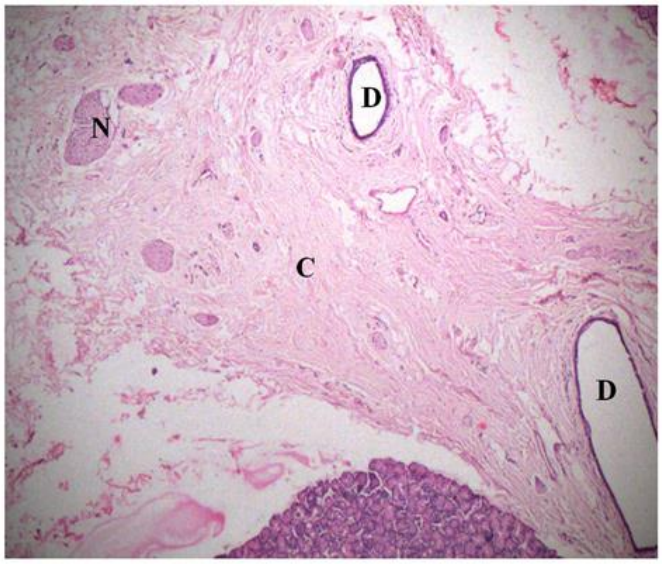

Fig-2: Photomicrograph of lacrimal gland of buffalo showing interlobular connective tissue (C) having interlobular duct (D) and nerve fibers (N). H and E x 40

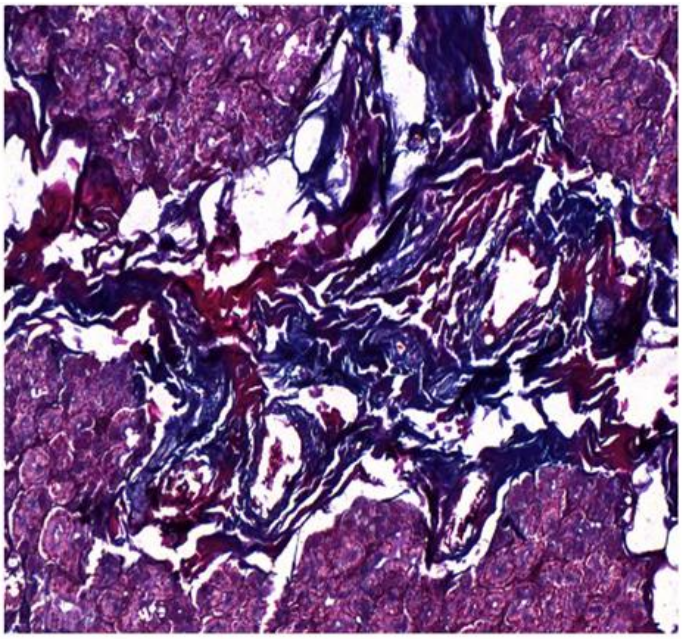

Fig-3: Photomicrograph of lacrimal gland of buffalo showing distribution of collagen fibers in the connective tissue of interlobular area. Crossman' s trichrome x 100

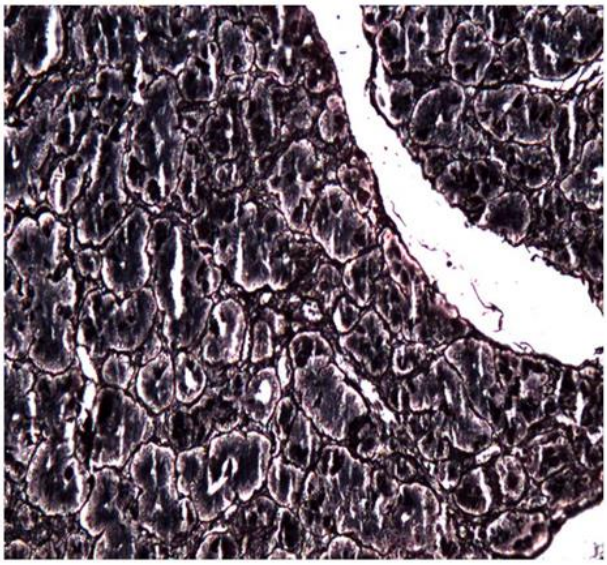

Fig-4: Photomicrograph of lacrimal gland of buffalo showing distribution of reticular fibers surrounding the glandular acini. Gomoni's method x 100

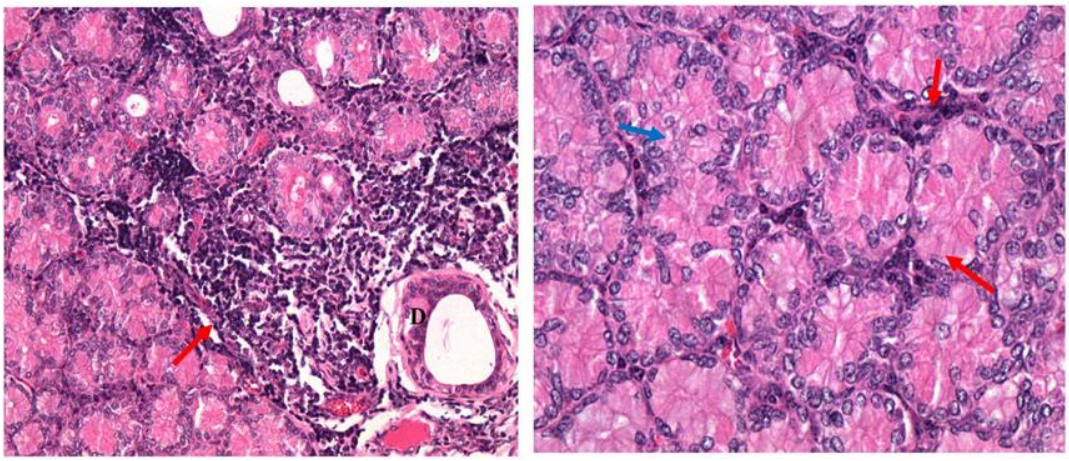

Fig-5 & 6: Photomicrograph of lacrimal gland of buffalo showing pyramidal shaped serous acinar cells surrounded by myoepithelial cells (blue arrow) and clusters of plasma cells (red arrow). H and E x 400

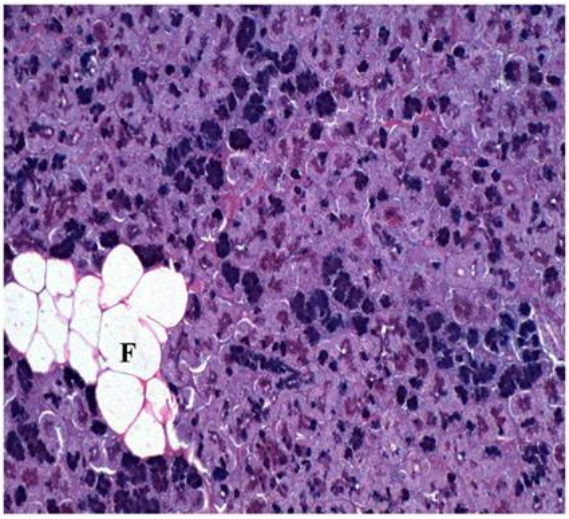

Fig-7: Photomicrograph of lacrimal gland of buffalo showing PAS positive activity of glandular acinar cells for both acdic and neutral mucosubstances. Notes absence of activity in clusters of fat cells (F). PAS AB x 100

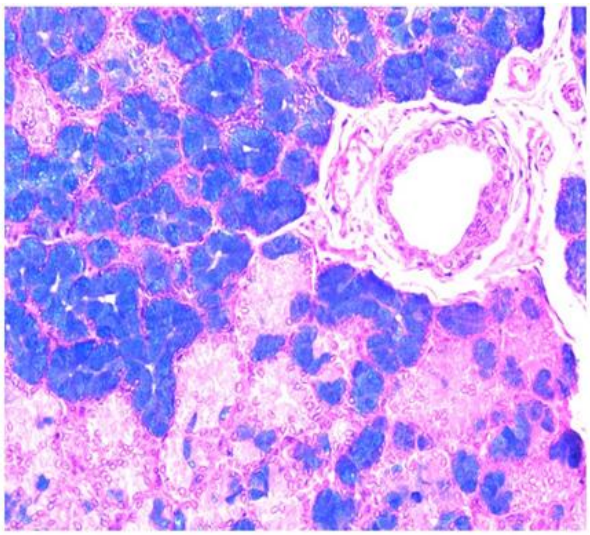

Fig-8: Photomicrograph of lacrimal gland of buffalo showing different types of reactions of the glandular acinar cells for Alcianophilic activity. AB x 100

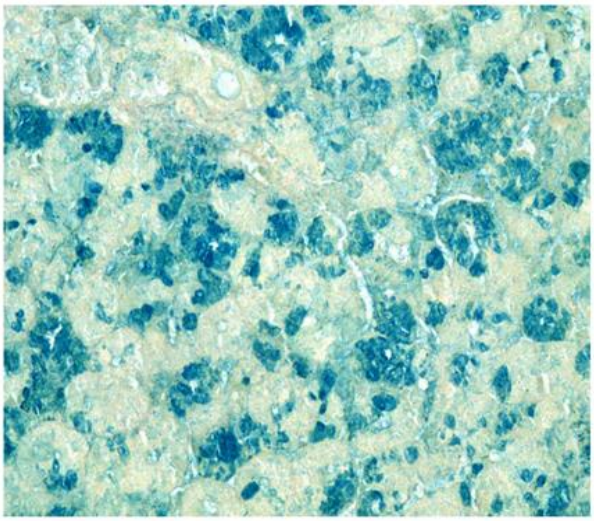

Fig-9: Photomicrograph of lacrimal gland of buffalo showing moderate to stong activity of the acinar cells to acid mucopolysaccharides. Colloidal method x 100

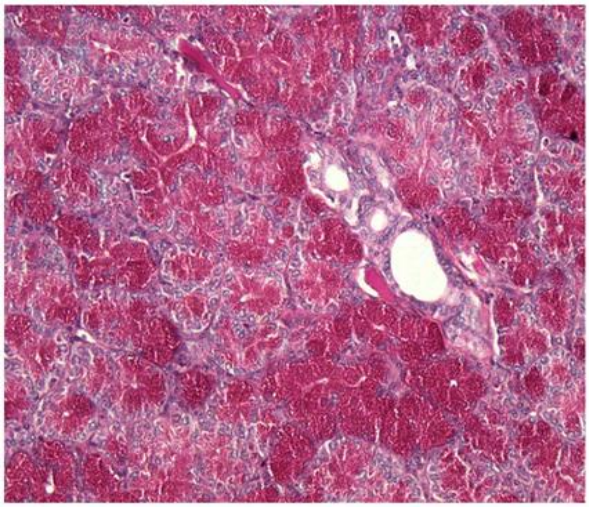

Fig-9: Photomicrograph of lacrimal gland of buffalo showing moderate to stong activity of the acinar cells for glycogen. Note the activity is absent in intralobolar ducts. McManus' PAS AB x 100

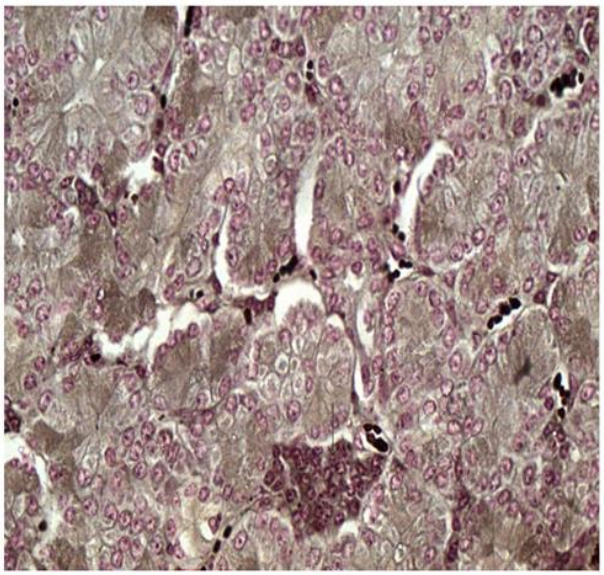

Fig-11: Photomicrograph of lacrimal gland of buffalo showing mixture of lightly stained serious acinar cells, comparatively darker mucous cells and darkly stained plasma cells (arrow). Fontana method x 200

The histochemical investigations of the LG revealed the different phase of secretory activities of the acinar cells for both acidic and neutral mucopolysaccharides as demonstrated by PAS-AB method (Fig-7) as reported in the alpaca [16]. However, only a few secretory cells containing slightly PAS positive granules (mucous cells) were observed in the roe deer [15]. In the present study, the neutral mucopolysaccharides were mainly present toward the apical portion of the acinar cells. The acini also showed strong Alcianophilic reaction indicating the presence of weakly sulfated mucosubstances, hyaluronic acid, sialomucins (Fig-8) and glycogen (Fig-9) as reported by Pinard *et al.*, [9] in the cattle and bison, Maala *et al*., [18] in Philippine buffaloes and Abbasi *et al*., [18] in the sheep. Klećkowska-Nawrot *et al*., [16] demonstrated the presence of slightly positive granules in mucous cells in the alpaca. Furthermore, Maala *et al*., [18] reported a weak reaction with Alcian blue (pH 1.0) in Philippine buffaloes. The differences observed in the reactivity of the granules in LG secretory cells between samples could be attributed to the various secretory phases of the same cells [29]. Similarly, moderate to mild activities of the acinar cells for mucin and colloidal iron were observed (Fig-10). In contrast, strong reaction for mucicarmine was reported in Philippine buffaloes [18]. Mucins were glycoproteins expressed by epithelial tissues and were classified as either secretory or membrane bound [30]. The lacrimal gland is being considered as a source of soluble and membrane-bound mucin [31]. It has been stated that ocular mucins possessed antimicrobial properties and play major role in dry eye syndromes [30]. The intra and interglandular ducts in this study revealed weak reactions for PAS, and Alcian blue as described in the sheep [7] and roe deer [15]. The presence of endocrine cells in the LG was not established using the Fontana-Masson method (Fig-11). However, the stain employed revealed lightly stained serous and heavily stained mucous acini cells of the glandular parenchyma, with intensely dark plasma cells within the interstitium. A study by Wood and Warren [32] in rabbit confirmed that lacrimal gland acinar cells produced endogenous prolactin-like substance which has a modulating influence on acinar cell activity as well as immune function of the lacrimal gland. Similarly, it has been proposed that that lacrimal gland integrity and function depend significantly on the action of androgens and the period of insufficient androgen levels could be the cause for primary lacrimal deficiency [33]. Androgens and estrogens modulate lacrimal gland secretion and lack of androgens causes reversible degenerative changes in lacrimal tissue, a decreased total volume of tears, and decreased protein content of tears [30].

CONCLUSION

The compound tubulo-acinar type of LG of the Indian buffalo was consisted of mixed distribution of acinar cells which were purely serous, mucous or mixed with variation in their histochemical characteristics. The basic histo-morphological data provided could be useful in comparative anatomy and clinical conditions related to the eye.

REFERENCES

1. Budras KD, Habel RE, Mulling CK, Greenough PR, Wunsche A, Buda S. Bovine anatomy, Schlütersche Verlagsgesellschaft mbH and Co. KG, Hannover, Germany. 2011.
2. Bacha Jr WJ, Bacha LM. Color atlas of veterinary histology. John Wiley & Sons; 2012 Jan 19.
3. Eurell JA, Frappier BL, editors. Dellmann's textbook of veterinary histology. John Wiley & Sons; 2013 Mar 19.
4. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional

unit in the pathophysiology of dry eye. Experimental eye research. 2004 Mar 1;78(3):409-16.
5. Walcott B. The lacrimal gland and its veil of tears. Physiology. 1998 Apr;13(2):97-103.
6. Shaker MM, AL-Obeady WF. Anatomical and histological study of the lacrimal gland of the adult male dog (Canis Familaris). GJBB. 2016;5:520-4.
7. Abbasi M, Karimi H, Gharzi A. Preliminary anatomical and histological study of lacrimal gland in Lori sheep. Veterinary Science & Technology. 2014 Jan 1;5(1):1.
8. Triveni T, Ashok P, Girish MH, Dileep Kumar D. Comparative histological studies of lacrimal gland in ruminants. Frontier Journal of Veterinary and Animal Science. 2017, 6: 1-4.
9. Pinard CL, Weiss ML, Brightman AH, Fenwick BW, Davidson HJ. Normal anatomical and histochemical characteristics of the lacrimal glands in the American bison and cattle. Anatomia, histologia, embryologia. 2003 Oct 1;32(5):257-62.
10. Mohammadpour AA. Anatomical characteristics of dorsal lacrimal gland in one humped camel (*Camelus dromedarius*). Journal of Biological Science. 2009, 8: 1104-1106.
11. Mohammadpour AA. Histochemistry of dorsal lacrimal gland in camel (Camelus dromedarius). Journal of Camel Practice and Research. 2011;18(1):131-3.
12. Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology.1968.
13. Crossmon G. A modification of Mallory's connective tissue stain with a discussion of the principles involved. The Anatomical Record. 1937 Aug 1;69(1):33-8.
14. Humason GL. Animal Tissue Techniques. 3rd edn, W.B. Freeman and Company, San Francisco, U.S.A. 1972.
15. Klećkowska-Nawrot J, Marycz K, Czogała J, Kujawa K, Janeczek M, Chrószcz A, Brudnicki W. Morphology of the Lacrimal Gland and Superficial Gland of the Third Eyelid of Roe Deer (Capreolus Capreolus L.). Pakistan Veterinary Journal. 2013 Apr 1;33(2).
16. Klećkowska-Nawrot J, Nowaczyk R, Goździewska-Harłajczuk K, Krasucki K, Janeczek M. Histological, histochemical and fine structure studies of the lacrimal gland and superficial gland of the third eyelid and their significance on the proper function of the eyeball in alpaca (Vicugna pacos). Folia morphologica. 2015;74(2):195-205.
17. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J. Pembrolizumab versus ipilimumab in advanced melanoma. New England Journal of Medicine. 2015 Jun 25;372(26):2521-32.
18. Maala CP, Cartagena RA, De Ocampo DG. Macroscopic, histological and histochemical characterization of the lacrimal gland of the Philippine water buffalo (*Bubalus bubalis*). Philippine Journal of Veterinary Medicine. 2007, 44: 69-75.
19. El-naseery NI, El-behery EI, El-Ghazali HM, El- E. The structural characterization of the lacrimal gland in the adult dog (Canis familiaris). Benha Veterinary Medicine Journal. 2016;31:106-16.
20. Shadkhast M, Bigham AS. A Histo-anatomical study of dorsal lacrimal gland in Iranian river buffalo. Veterinary Scan. 2010, 5: 1-5.
21. Wang YL, Tan Y, Satoh Y, Ono K. Morphological changes of myoepithelial cells of mouse lacrimal glands during postnatal development. Histology and Histopathology. 1995, 10: 821-827.
22. Helen PM, Darlene AD. Myoepithelial cells: their origin and function in lacrimal gland morphogenesis, homeostasis, and repair. Current Molecular Biology Reports. 2015, 1: 115-123.
23. Aitken ID, Shurvashe BD. Plasma cells in vertebrate paraocular glands. Archives of Allergy Applied Immunology. 1977, 53: 62-67.
24. Gillete TE, Allansmith MR, Greiner JV, Janusz M. Histologic and immunohistologic comparison of main and accessory lacrimal tissue. American Journal of Ophthalmology. 1980, 89: 724-730.
25. Knop E, Knop N. Lacrimal Drainage–Associated Lymphoid Tissue (LDALT): A Part of the Human Mucosal Immune System. Investigative ophthalmology & visual science. 2001 Mar 1;42(3):566-74.
26. Bron AJ, Seal DV. The defenses of the ocular surface. Transactions of the Ophthalmology Society UK. 1986, 105: 18-25.
27. Coyle PK, Sibony PA. Tear immunoglobulins measured by ELISA. Investigative Ophthalmology and Visual Science. 1986, 27: 622-625.
28. Sullivan DA. Ocular secretory immune system: lacrimal gland and conjunctiva. In: Mucosal Immunology and Ocular Disease. (eds Zierhut M, Forrester JV) Aeolus Press, 2000.
29. Krochmalska L. Histological and histochemical picture of the lacrimal gland in guinea pig. Acta Theriogenology. 1976, 21: 31-36.
30. Davidson HJ, Kuonen VJ. The tear film and ocular mucins. Veterinary Ophthalmology. 2004, 7: 71-77.
31. Corfield AP, Carrington SD, Hicks SJ. Ocular mucins: purification, metabolism, and functions. Progress in Retinal and Eye Research. 1997, 16: 627-656.
32. Wood RL, Warren DW. Prolactin and prolactin receptors in the lacrimal gland. Experimental Eye Research. 1999, 69: 213-226.
33. Azzarolo AM, Wood RL, Mircheff AK, Richters A, Olsen E, Berkowitz M, Bachmann M, Huang, ZM, Zolfagari R, Warren DW. Androgen influence on lacrimal gland apoptosis, necrosis, and lymphocytic infiltration. Investigative Ophthalmology and Visual Science. 1999, 40: 592-602.