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ANATOMICAL AND HISTOLOGICAL STUDY OF THE LACRIMAL GLAND OF THE ADULT MALE DOG (*Canis familaris*)

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ABSTRACT
The aims of this research have been performed to investigate the anatomical, histological structure and histochemical properties of the lacrimal gland which are responsible for lacrimation in adult male dog (*Canis familaris*). This study includes 10 heads of healthy adult male dogs aged between (24–36) months, body weights ranged between (18-24) kg. Twenty samples (10 right +10 left) of each lacrimal gland in dog, taken immediately after (15-20 min) and keep in 10% formalin for histological investigation. The anatomical results showed that the lacrimal gland in dog was located dorsolaterally underneath the medial surface of the orbital ligament. Its boundaries are is the frontal bone dorsally, zygomatic bone ventrally. The mean length, width, and thickness of right lacrimal gland in dog were (1.3 ±0.14mm), (2.32 ±0.049mm), (0.51 ±0.039mm) respectively, also of left side (1.4 ±0.07mm), (2.42 ±0.072mm), (0.49 ±0.032mm) respectively. There was significant difference on both sides at ($p \leq 0.05$). Histological sections of lacrimal gland in dog were compound mixed tubuloalveolar, acini gland. The glandular lobules were measured (1245.63 ±56.15µm). The lacrimal gland composed of mucous and serous secretory units; each was measured about (33.5 ±0.11 and 46.4 ±0.15µm) in diameter. The mean diameter of intralobular ducts was measured (19.83 ±0.05 and 20.5 ±0.11 µm) at right and left in order. The mean diameter of interlobular ducts was measured (39.65 ± 0.55 and 40.97± 0.51 µm) at the right and left side respectively. The histological results revealed that the main ducts, which conveys the secretion into the inner surfaces of the eyelids, was lined by stratified cuboidal cells showed sub epithelial aggregation of lymphocytes and plasma cells. In conclusion topographic anatomical location of lacrimal glands in dog seemed the location at the dorsolateral region of the orbit, but situated under beneath the orbital ligament due to absence of orbital ridge. The lacrimal gland of dog smaller in all anatomical parameters. The lacrimal gland in dog histological is the compound tubuloalveolar gland with seromucous acini, but the serous acini in dog more. The histological study results revealed no intercalated duct in lacrimal glands of dog.

KEYWORD: Lacrimal, Serous acini, Mucous acini, Histochemical, Ducts, Dog.

INTRODUCTION

The gland which responsible for lacrimal fluid production (tear) components includes the lacrimal gland (Prince *et.al*, 1960).The glands responsible for lacrimal fluid production are distributed as three groups of major exocrine glands. The main lacrimal gland located dorsolateral aspect of the orbit (Getty, 1975; Sakai, 1989).Generally the lacrimal fluid components helps maintain the cornea via spreading the major source of proteins and electrolytes act as nutrients of the cornea and lubricate and moistured the bulbar and palbebral conjectiva in addition to protect the eye from pathogen (Hirayama *et al*., 2013).The lacrimal gland located in the dorsolateral region of orbital cavity dorsal to the eye ball. The shape of the lacrimal gland is flattened due to the location between the bony orbit and eyeball (Prince *et al.*, 1960; Getty, 1975). It's located within the periorbital fascia at the level of the dorsolateral aspect of the eye ball surround by the frontal bone. So the ventral surface was slightly concave, fitting over the eyeball and its dorsal surface convex because their positions under beneath the frontal bone (Getty, 1975).In dog lacrimal gland is small, flat lobular structure lying on the medial side of the orbital ligament within the periorbital. Small ducts that cannot be seen only in microscopically are excreted their secretions into the conjunctival sac at the dorsal fornix (Bruce *et al*., 2004; Evans *et al*., 2010). Generally in all domestic animals the ventral surface of lacrimal gland lay on the caudodorsolateral surface of the eyeball from which it was separated by the periorbital. The rostromedial margin of the gland was situated sagitally to the supraorbital process (Saber *et al*., 1987).The anatomical parameters of lacrimal gland in Lori sheep the mean weight (1.48 ±0.3 gr), the mean length (26.95 ±0.37 µm) and the mean width (20.11 ±0.3 µm) with mean thickness (3.58 ±0.7 µm) (Abbassi *et al.*, 2014).The weight of the lacrimal gland ranged between 1.95 and 2.49gm. As a single unit the gland measured 55 µm in length, and 20 µm in width. The length of the cranial lobe was 35mm, and the width 20 µm. The caudal lobe was 20 µm in length and width. The thickness of the gland varied between 5 µm in the middle of the cranial lobe and 2 µm in the most lateral aspect of the caudal lobe and the most medial part of the cranial lobe (Ibrahim *et al*., 2010). Histologically, the lacrimal glands are consisting of secretory units called acini that secreted mucin passed via these ducts to surface of conjunctiva (Dugan. 1992; Cormack, 1996). The secretory glandular terminals consisted of tubuloacinar units and acini arranged around the tubular segments, according to the results reported by (Cormack, 1996). In dog lacrimal gland

is tubuloacinar gland on the episclera in the superior temporal orbit. These glands produce approximately 60% of the serous portion of tears in dogs (Bruce *et al*., 2004; Evans *et al*., 2010).The lacrimal gland is a serous acini lobulated gland, which has comprised of cuboidal epithelium. The secretary variations are observed in different domestic animals. In swine, the secretion is mucous in nature. The cells are showing mucin reaction in sheep, goat and dog (Menaka *et al*., 2015; Leeson *et al*., 1971). The glandular stroma constituted from the epithelial components (ducts and secretory units) which principally compose the glandular parenchyma. Conjunctival tissue, including blood vessels and nerve fibers (Cormack, 1996).The interstitium was found to be constituted by some collagen fibers separating the glandular lobes, myoepithelial cells, plasma cells and lymphocytes, as previously reported by others (Dugan, 1992). There were three types of secretory cells are observed in this gland; serous, mucous and seromucous cells. In sections obtained from the ovine lacrimal gland, serous and mucous cells were both identified and thus the gland is a mixed seromucous gland. The mixed seromucous glands have also been reported in many mammals, including the pig, horse, goat, and hamster. Lacrimal gland of the canine is a mucous gland while in the rat, it is a serious gland. In the sheep the majority of acini contain mixed serous, seromucous and mucous cells (Gargiulo *et al*., 1999).

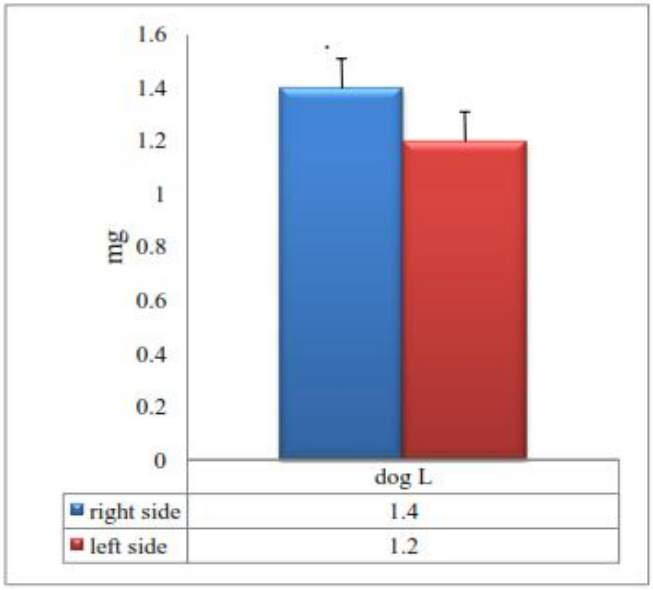
MATERIALS & METHODS

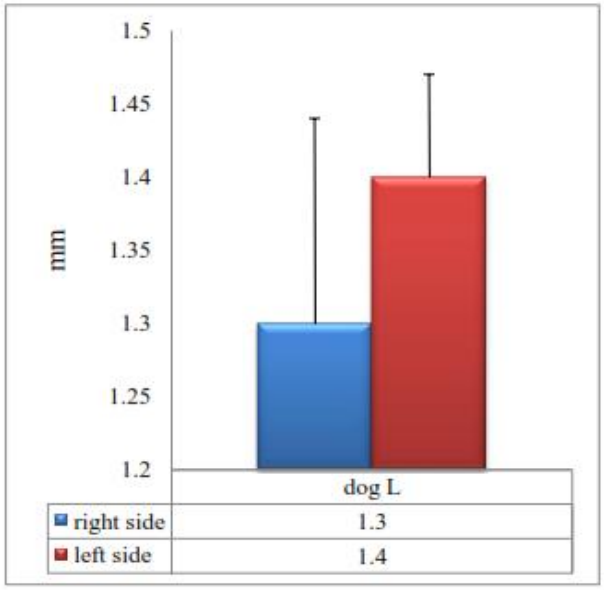
This study carried out on 10 heads of adult male healthy dog aged between (24–36) month body weights ranged between (18-24) kgs, mean and SE were calculated (21.5 ±1.3), bought from the local market by dog owners in Babylon governorate. The dogs were anesthetized by using a mixture of xylazine hydrochloride 2% at a dose of 0.05 mg/kg B.W. and ketamine hydrochloric 10% at a dose of 3mg/kg B.W. was administrated intramuscularly to provide general anesthesia (Ivany and Muir, 2004). During removal of specimens the anatomical description was done carefully. The specimens were fixed in 10% formalin for 48 hours for histological processing (Fraenkel-Conrat *et al*., 1949, 1948a, 1948b). Histological and histochemical techniques include: Fixation the specimens ($1cm^3$) were fixed immediately after dissection in 10% formalin at room temperature (37-38ºC) for light microscopic study. Dehydration by using up graded series of ethanol (Scharlau) (70%, 80%, 90% and 100% two changes) two hours for each concentration to remove water from the histological specimens. Clearing the clearing processes have been made by xylene (Scharlau) for half hour/two changes (Luna, 1968). Embedding the following step includes infiltration by embedding of the specimens in paraffin wax of melting point 58-60ºC. Paraffin must be fully molten to infiltrate the tissue effectively. This step continuous by using three bathes of molten paraffin in the oven at 60ºC, each bath is continuous for one and half hour. Blocking the tissue samples are placed in small containers (blocks) already filled with molted paraffin and orientation of the tissue is important in order to determine the proper surface for sectioning to get paraffin blocks (Luna, 1968). Sectioning the paraffin blocks were sectioned by using semi digital rotary microtome to get paraffin sections measured (6) micrometers thickness fixed on class slides coated with egg albumin. Staining techniques H&E to determine the general histological features and parameters of different studied glands (Luna, 1968). PAS for the demonstration of basement membrane, glycoprotein, mucopolysaccharide, Masson's Trichrome for demonstration of Collagen and muscle fibers. Van Gieson for demonstration of collagen and muscle fibers. Alcian Blue for demonstration of mucosubstance (ph 2.5). Alcian Blue + PAS to determined the acidic mucin and neutral mucin in the acini of the gland.

RESULTS

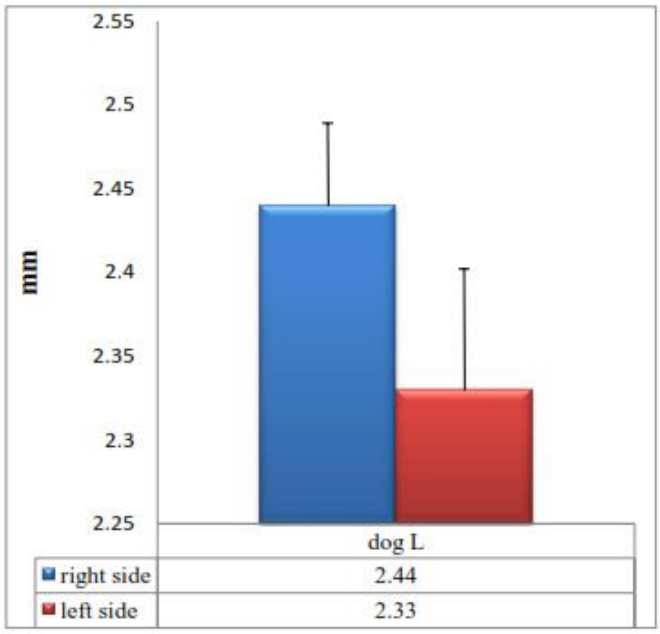
In dog the lacrimal gland located dorsolateral underneath the medial surface of the orbital ligament (fig. 1). Its boundaries are upper is the frontal bone, lower zygomatic bone. In dog the weight of the lacrimal gland in right was (1.4 ±0.11 mg) and the left one (1.2 ±0.11 mg). There was significant difference between right and left side in dog at level ($p<0.05$) (Histogram-1). The other anatomical parameters of lacrimal gland in dog the mean length right lacrimal gland was (1.3 ±0.14 µm) and (1.4 ±0.07 µm) of the left side (Histogram- 2). The measurements in dog the width mean of the right side of lacrimal gland (2.32 ±0.049 µm) and in the left side (2.42 ±0.072 µm). (Histogram- 3).The mean thickness of the lacrimal gland in dog in right side were (0.51 ±0.039 mm) and the left side (0.49 ±0.032 mm) (Histogram- 4).

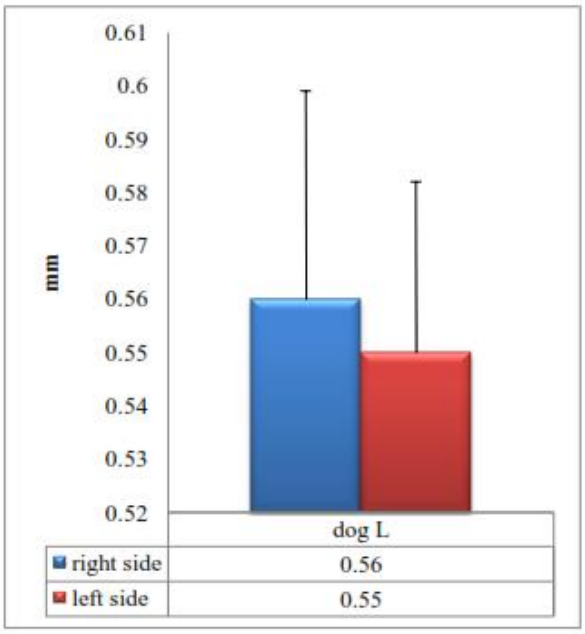
In dog, lacrimal gland was compound tubuloalveolar, acinar gland, composed of several lobules that separated by inter lobular loose connective tissue septae, each glandular lobules was measured (1245.63 ±56.15µm) (fig. 2). It composed of mucous and serous secretory units; each was measured about (33.5 ±0.11 and 46.4 ±0.15µm) in diameter respectively (fig. 3). The mucous alveolar cells were tall columnar cells with darkly stained round nuclei in basal position while the serous acinus cells were low cuboidal cells and their nucli were centrally positioned (fig.4). Myoepithelial cells were laid between the gland epithelium and the basal membrane (fig. 5). The thick layer of connective tissue was laying between the secretory units composed only collagen bundles and showed no smooth muscles (fig. 6). the glandular duct system showed no intercalated ducts and started with intralobular duct, the intralobular duct lead to interlobular duct within septum both duct were lined with low cuboidal cells , the mean diameter of intralobular duct were measured (19.83 ±0.05 and 20.5 ±0.11 µm) right and left respectively. (fig. 7), The interlobular duct lead to main duct the convey secretion to posterior surface of eyelid which lined with stratified cuboidal cells. The mean diameter of interlobular duct was measured (39.65 ± 0.55 and 40.97± 0.51 µm) right and left respectively (fig 8).The secretory cells of lacrimal gland contained positive PAS and positive Alcian blue cytoplasmic granules (fig. 9).





Histogram 1: showing the weight of the lacrimal gland (L) in dog/ mg
Histogram 2: showing the length of the lacrimal gland (L) on dog .





Histogram 3: showing anatomical parameter of the width of the lacrimal gland (L) in dog
Histogram 4: showing the thickness of the lachrymal gland (L) in dog

DISCUSSION

The lacrimal gland is very small structure and the characterization of its appearance and morphology will help in recognition of any abnormality in the gland. The results of this study revealed that the location of the lacrimal gland in dog its location characterized by more laterally at the medial surface of the orbital ligament. The weight of lacrimal gland in dog was distinguished smaller in both sides. On the other hands, there were no significant differences between left and right lacrimal gland weight and thickness of animal because asymmetry of the gland could be an important indicator for unilateral diseases. The present finding revealed that the lacrimal gland of the dog was tubuloalveolar and acinar gland (serous & mucous), this result is similar with result of (Mohammad pour, 2011; Schechter *et al*., 2010; Ding *et al*., 2010; Klećkowska- Nawrot and Dzięgiel, 2007, 2008; Gargiulo *et al*., 1999 and; Cormack, 1996; Hirsch-Hoffmann,1976), while the present result disagree with (Gargiulo,*et.al*.1999) who mentioned that the mixed seromucous glands have reported in pig, horse, goat, and hamster, while in the rat, it is a serous gland and in the sheep the majority of acini contain mixed serous, seromucous and mucous cells. On other hand the present results showed that the mucous alveoli had larger diameter than the serous acini in addition to that, the lacrimal gland of dog was predominantly mucous type, this suggested that this gland has a feature of mucous gland, this similar with result of

(Menaka *et al.*, 2015) in swine, sheep, goat and dog, while (Dugan. 1992; Cormack, 1996; Bruce *et al*., 2004) and (Evans *et al*., 2010) mentioned that, the predominant serous type of lacrimal gland that produce approximately 60% of the serous portion of tears in dog, while (Aldana, *et al.,* 2002), (Kühnel *et al.*,1979) in pig, and (Mohammad pour, 2008) in one-humped camel revealed that the lacrimal glands in pig and one humped camel are serous, mucous and mixed. On the other hand the variation in the type of glandular secretion is beyond to individuals. It was proven that the volumetric percentage of secretory parenchyma (acini and tubule) of the lacrimal gland was larger than that of the superficial gland of the third eyelid (Helper *et al*., 1974). The present result revealed columnar epithelium lined the mucous acini while the lining cells of serous acini was cuboidal cells, those surrounded by myoepithelial cells, such observation is recorded by (Menaka *et al*., 2015; Klećkowska *et al.,* 2008). The interlobular connective tissue was thick layer of irregular collagen bundles showed no smooth muscles which lacked muscle fibers that substituted by the presence of myoepithelial cells around the secretory units (Cormack, 1996; Dugan, 1992; Klećkowska *et al.*, 2008). whom refereed for collagen fibers separating the glandular lobes, myoepithelial cells, plasma cells and lymphocytes, as previously reported by others. The present finding revealed no intercalated ducts in the lacrimal gland of dog the duct system was started with intralobular such result agree with (Sakai, 1989; Hirsch-Hoffmann, 1976; Alexander *et al*., 1973), while this result is incompatible with result of (Kühnel *et al*., 1979) in pig who referred for prominent duct system started by intercalated ducts which lined by low cuboidal epithelium and surrounded by myoepithelial cells. Also the lining epithelium of interlobular duct in lacrimal gland of dog was similar to that observed in one-humped camel but goblet cells are present among epithelial cells of interlobular ducts also such records was observed in the lacrimal gland of Lori sheep. (Mohammad pour, 2008) and (Alexander, *et al*., 1973) in rat. On the other hand, in ovine the excretory ducts which open into the front fornix of the upper eyelid conjunctiva lined with a stratified columnar epithelium containing many goblet cells (Lorber, 2009).

CONCLUSIONS

1. Topographic anatomical location of lacrimal glands in dog seemed the location at the dorsolateral region of the orbit, but situated under beneath the orbital ligament due to absence of orbital ridge.
2. The lacrimal gland of dog smaller in all anatomical parameters.
3. The lacrimal gland in dog histological is the compound tubuloalveolar gland with seromucous acini, but the serous acini in dog more.
4. The histological study results revealed no intercalated duct in lacrimal glands of dog.
5. Myoepithelial cell found in the parenchyma of the lacrimal gland in dog around the acini and the intralobular duct.

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