Morphohistochemical, Immunohistochemical and Histoenzymatic study of Mandibular Salivary Gland in both sex in Awassi Sheep

Hiba M. Abd Alrahman[1]*, Azal N.B.Al-Nusear[1], Shireen J. Khaleel[1], Rana Khalaf Abdulsamad[1], Ashraff W.Abdulrazaq[2] And Nadhim A. Shehan[1]

Abstract

Background: Salivary glands play an important role in the lubrication of food and protection of the oral mucosa through the production of saliva. Salivary glands are mainly classified into minor and major salivary glands. The parotid, mandibular, monostomatic and polystomatic sublingual, and zygomatic glands Enzyme histochemistry serves as a link between biochemistry and morphology. **Aim:** The objective is to gain a deeper understanding of the morphological structure of the mandibular salivary gland and provide additional information about the histoenzymatic and immunohistochemical structures present in this gland. **Methods:** Fifteen head of healthy animals was used to carried the morphistological and histochemical studies of general structures of the mandibular gland (MSG) and the immunohistochemical and histoenzymatic study was carried to demonstrated the macrophage localization and Alkaline phosphatase deposition respectively. The H& E stain with PAS, Alcian blue and Masson's Trichrom stain was explained the (MSG) in sheep was consisted of thick capsule of connective tissue and septa that divide the gland into lobes and lobules, mainly consisted of collagen fibers. **Results:** The secretory unit composed of predominant mucus acini with diameter (55.62±11.74381µm)and serous acini with diameter (33.95±12.3323µm). the systemic ducts were consisted from intercalated duct, striated duct, interlobular duct and interlobar duct with diameters (63.96±13.6029 µm, 183.34±19.8125 µm, 181.28±40.9417 µm and710.7000±289.9592 µm) respectively. of the PAS stain have positive reaction in the epithelium of duct and connective tissue, AB stain was gave obvious staining in the interlobar duct. CD 68PG M1 antibody was used and give positive reaction with weak intensity for macrophage. The Alkaline phosphatase gave moderate positive blue to green color around the acini and in the intercalated and striated duct. **Conclusion:** The morphological and histochemical structures of the mandibular gland of sheep was consisted of thicker collagenous connective tissue capsules, delicate septa divided it into prominent lobes and lobules, With large mucous acini and small serous acini, and the its ducts have diameter gradually increased, at the beginning the small diameter of intercalated duct and ended with the large interlobar duct.

[1]Department of Anatomy and Histology, College of Veterinary Medicine, University of Basrah, Iraq
[2]Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Basrah, Iraq

Corresponding Author*: Hiba M. Abd Alrahman, Department of Anatomy and Histology, College of Veterinary Medicine, University of Basrah, Iraq, Email: heba.mohammed@uobasrah.edu.iq, ORCID ID: https://orcid.org/0000-0003-3573-9414

Keywords: Histological, Immunohistochemical, Histoenzymatic , CD68 PG M1, Alkaline phosphatase, Mandbular salivary gland, sheep.

Introduction

Salivary glands play an important role in the lubrication of food and protection of the oral mucosa through the production of saliva, which plays an important role as an antimicrobial agent and helps in heat loss during painting (Eubanks and Woodruff 2010). Salivary glands are mainly classified into minor and major salivary glands. The parotid, mandibular, monostomatic and polystomatic sublingual, and zygomatic glands are categorized as major salivary glands, while the labial, buccal, molar, lingual, and palatine are grouped under the minor salivary glands in the canine species (Evans and de Lahunta, 2013; Gioso and Carvalho,2005). The submandibular salivary gland being one of the major salivary glands contributes to a substantial amount of saliva secreted into the mouth. Saliva contains water, various enzymes, mucopolysaccharides, and lubricating glycoproteins. The gland has an important role to provide lubrication for eating and vocalization, aid digestion, and the supply saliva for pH buffering (Moghaddam et al., 2009). Enzyme histochemistry serves as a link between biochemistry and morphology. It can be used to locate many enzymes, including phosphatases,dehydrogenases, and esterases. Alkaline phosphatase (AKPase) is associated with the ionic exchange across the membrane.(Hardonk & Koudstaal, 1979).The mandibular gland was recognized by the ovoid shape, the smooth surface and located at the level of the digastric muscle, and surrounded by the medial retropharyngeal lymph node medially, the linguofacial vein lateroventrally and the maxillary vein laterodorsally (Weidner et al., 2012). parenchymal unit of the salivary gland is referred to as a (acinus) that is composed of pyramida secretory cells arranged around a central lumen and surrounded by myoepithelial cells. The lumen of the acinus leads to a duct system. Acini are made up of either serous or mucous cells, some of the mucous acini have a cap of serous cells called demilunes(Delporte et al., 2016).The mandibular salivary gland is covered by a fibrous capsule of dense connective tissue and it is generally a compound branched tubuloacinar gland (Dellman and Brown, 1981). Due to the dearth of information of microstructures on the mandibular salivary gland in sheep so our study focused on the morphohistology, histochemistry, immunohistochemistry and histoenzymat studies of mandibular salivary gland in sheep. By integrating, structural, connective tissue, cellular data and enzymatic activity, this work seeks to provide baseline information that may aid in the future morphological, pathological, immunological and histoenzymatic investigation in the domestic mammals.

Aim of the study: To understand the morphohistological architectures of the mandibular salivary gland and give more details about the histoenzymatic and immunohistochemical structures in this type of the gland.

Materials and methods

Sample collection

The current study was conducted to College of Veterinary Medicine in Basrah University. The samples of mandibular salivary gland were used in this study collected from fifteen heads of healthy adult Awassi sheep randomly collected from both sexes and from both side left and right from the slaughter house in Basrah Governorate to study the morphohistological, histochemical, histoenzymatic and immunohistochemical structures of the mandibular salivary gland in sheep.

Histological and histochemical observation

For histological study the collected samples of mandibular salivary gland from sheep was fixed in 10% buffered formalin, Ascending concentrations of ethyl alcohol were used for dehydration, cleared in xylene and impregnated with paraffin wax (Drury and Wallington, 1980), then stained with Harris haematoxyline and eosin stain for general histological studies(Harris, 1996), Periodic acid-Schiff (PAS) stain for detection of neutral mucopolysaccharides(Mc Manus, 1946), and Alcian blue stain (pH 2.5) for detection of acidic mucopolysaccharides(1950).

Histoenzymatic study

Histoenzymatic study was obtained by studying the activity of Alkaline phosphatase. Within the gland.

Statistical analysis

Five animals were used to understand the variances in the diameter of serous and mucous acini, intercalated, striated, interlobular and interlobar ducts of mandibular salivary gland in sheep, the statistical software program (SPSS for Windows version 22, USA) was used to analysis the mean and standard error were used to display the

results., the data were statistically analyzed by One-Way ANOVA (Tukey HSDa) with multiple comparison tests at ($P \leq 0.05$) difference was considered significant.

Result

In this study the mandibular gland in Awassi sheep was irregular lobulated shape with pink color and softness surfaces, located at the base of the mandible contact to the medial surface of it and rested between the inter mandibular spaces, partially covered by the distal part of parotid gland and extended from the wing of atlas caudally (Figure 1).

Histological findings

The histological structures of the mandibular gland revealed by the stroma and parenchyma; the stroma consisted of a surrounding dense connective tissue capsule from which the septa was projected to the parenchyma of the gland and divided the gland into lobes and lobules. These septa carried out the blood vessels, nerves, and the main ducts of the salivary gland (Figure 2). The mandibular gland was compound tubulo-Acinar, these acini composed of mucus acini that was predominant than serous acini and demilunes. The mucous acini, which was pale-stained, appeared as the foam cytoplasm lined by the cuboidal cells with round nuclei located near the base of the cells and had a wide lumen, while the serous acini, which was pale-purple in color, had a pyramid shape of cells, and their nuclei were more centrally within the cells, and their lumen was narrow. The demines were arranged as a serous crescent surrounded the mucus acini (Figure 3 and figure 4). The myoepithelial cells with spindle shape surrounding the mucus acini and intercalated duct (Figure 5). The duct system of parenchyma was consisted of the intercalated duct with different shapes, which was lined by low simple cuboidal epithelium that received the secretion from the acini and reached it to the striated duct, which appeared large in size and lined by the columnar epithelium and then went empty to the larger duct surrounded by connective tissues within the septa and lobules that were called the interlobular duct these ducts are lined by the simple columnar epithelium (Figure 6) and the interlobar duct that is located in the septa and lined by a stratified columnar to cuboidal epithelium with large lumen and pale goblet cells. (Figure 7).

Statistical analysis:

Statically, there were significant differences in the diameter of the secretory unit and ducts at level ($P \leq 0.05$), the acini was larger in mucus acini (55.62±11.74381μm) when compared with serous acini (33.95±12.3323μm). The diameters of the ducts are increased gradually as they go toward the oral cavity. It began with the intercalated duct that has the smallest diameter (63.96±13.6029 μm), then the striated duct (183.34±19.8125 μm), interlobular duct (181.28±40.9417 μm), and finally the interlobar duct that appeared to have the largest diameter (710.7000±289.9592 μm). (Table 1) and (Figure 8).

Histochemical structures:

PAS reaction: The serous acini gave a positive reaction with a strong magenta color when compared with the mucus acini that gave a mild reaction with a light purple color. The collagen fibers and glycoproteins in the connective tissue in the capsule and around the basement membrane, acini, luminal epithelium of intercalated and striated ducts, and in the interlobar septa gave a strong positive reaction with pink- magenta color (Figure 9). Alcian blue : The mandibular salivary gland stained with Alcian blue revealed prominent mucus acini that gave positive light blue to green staining indicated the presence of acidic mucopolysacharides with serous demilunes surrounding the mucus acini and the interlobar duct was filed with secretion and gave positive color more than other ducts.(Figure 10). The Masson's trichrome stain: The collagen fibers were abundant in the capsule and interlobar septa of the gland and with delicate spars within lobules showing dark blue staining while the muscle fibers and the ducts appeared red to pink color and myoepithelial cells showed as a red spindle shape (Figure 11).

Immunohistochemical findings:

The macrophage that stained with CD68 PG M1 antibodies giving positive reaction with weak intensity; these cells can be shown in the connective tissue and through the glandular epithelium closely surrounding the secretory units and ducts. (Figure 12).

Histoenzymatic finding:

The salivary gland in sheep produced the large amount of saliva that gives a strong positive reaction to the alkaline phosphatase staining. The result shows deposition of this enzyme was more prominent around the acini and within the epithelium of striated ducts with dark brown staining than other ducts that stained with light brown color. The

spindle shape of myoepithelial cells were more visible around the acini and all the ducts of that involved in the squeeze the saliva during contraction and reach it to the oral cavity.(Figure 13) and (Figure 14).

Discussion

In this study the mandibular salivary gland in sheep situated in the under the parotid gland obliquely and rested at the angel of mandible between the intermandibular space this finding was agreement with reports in (Nourinezhad ei a;., 2021) in water buffalo and (Ibrahim et al., 2016) camel, ox, sheep and goat and (Nawar and El-Khaligi, 1977) in camel. It was irregular in shape, pale to pink in color and less compact than other salivary gland the results was similar ((Al-Sadi, 2013) in cattle, (Nawar, 1980 and Nickel et al., 1973) in sheep but dissimilar with ((Tadjalli et al., 2002) in dogs that report the glands have irregular triangle shapes may be differs according to species-specific , Sisson and Grossman (1975) described the color of the glands as bright yellow in general domestic animals. The lobulation of the gland was more prominent because the thick structures of septa that penetrated the gland and surrounding the parenchymatous tissue and it corresponded to the finding to (Nickel et al., 1973; Dehghani et al., 200) in sheep Rauf et al. (2004) in goat, Al-Sadi (2013) in cattle, (Gaber et al., 2022) reported the parenchymatous tissue of the mandibular gland in dogs is very dense and compact, while the supporting tissue is very weak, resulting in indistinct lobulation of the gland, the result showed the mandibular gland covered by thick connective tissue capsule predominant of collagen fibres for protection and supporting this agreement with ((Ibrahim et al., 2016) in camel and disagreement with his findings in sheep, goats, and ox in which the capsule consists of thin connective tissue. Also disagreement with (Dehghani et al., 2000a) in sheep. The parenchyma consisted of predominate mucous acini and less serous acini with few serous demilunes this result was agree with the (Rauf et al., 2004) in goat, (Adnyane et al., 2010) in deer and (Gaber et al., 2020) in dog that essential to lubricating the diet and provid covering and protection to the oral cavity and formed the bolus. in present study the intercalated duct was lined by simple cuboidal epithelium that similar as reported in the (Shackleford and Wilborn 1970) in calf, (Khalil, 1989) in camel to facilities the collection of saliva and secret bicarbonates and absorbed chloride , the striated duct was lined by simple columnar epithelium this finding corresponded to the(Rauf et al., 2004) in goat and (Ibrahim et al., 2016) camel, ox, sheep, and goat for active transport. The interlobular duct was lined by the simple columnar epithelium this agreement with result in (Nawar and EL-Khaligi , 1977) in camel, (Shackleford and Wilborn, 1968) in bovine, (Rauf et al. ,2004) in goat and (Jian etal., 2010) in pig, but the interlobar duct was lined by a stratified columnar epithelium with goblet cells that related to the result in camel in the previous studies. Myoepithelial cells around the acini and intercalated duct this agreement with (Ikpegbu et al., 2014) in rat. The histochemical finding of carpohydrate, mucopolysacharides and collagen fibers were gave positive reaction and this finding agreement with (Yang et al., 2019) in sheep and (Chen etal., 2025) in camel. The macrophage that dominant innate immune cells essential for homeostasis, tissue remodeling and immune surveillance, the CD68 PG M1 give wake positive staining for macrophage .Weak to moderate reaction of Alkaline phosphatase in the acini and more staining was in the interlobar duct this finding was similar to the (Singh &Singh , 2014) in buffalo.

Conclusion

The morphological and histochemical structures of the mandibular gland of Awassi sheep was consisted of thicker collagenous connective tissue capsules, delicate septa divided it into prominent lobes and lobules, mixed gland. The results of this paper was revealed there was significant variation in the diameter of the secretory units (acini) in the mandibular salivary gland of sheep and its component from the ducts, the mucous acini showed have more diameter with light foamy shape than the serous acini, while the intercalated duct have the smallest diameter with low simple cuboidal epithelium, while the interlobar duct have the larger diameter with stratified columnar to cuboidal epithelium with goblet cells. The presence of macrophages giving positive reaction to the CD68 PG M1 antibodies through the connective tissue and ducts in compared with animals . Alkaline phosphatase staining the saliva that more visible with dark brown color in striated ducts.

Research Ethics Committee Approval

None, the animals was taken from the slaughter house.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Data availability

All data available in the manuscript.

Abbreviations

None

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Tables

Table 1. Diameter of the acini and ducts in the mandibular salivary gland in sheep:

Mucus acini	Serous acini	Intercalated duct	Striated duct	Interlobular duct	Interlobar duct
55.62±11.74381C	33.95±12.3323C	63.96±13.6029C	183.34±19.8125B	181.28±40.9417B	710.7000±289.9592A

*A, B, C significant differences in the diameter of the mandibular salivary gland in sheep at P ≤0.05 value.

Figures

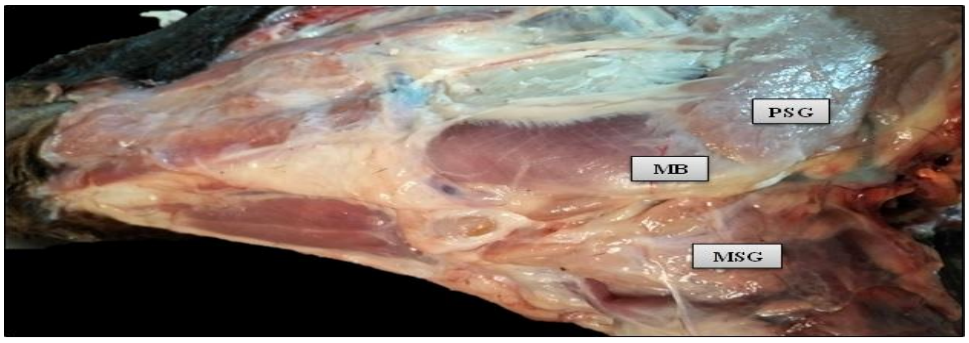
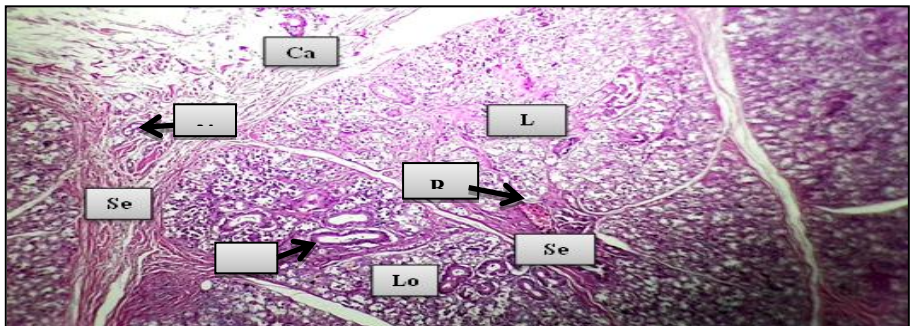


Figure (1) Morphological structures of mandibular salivary gland in sheep show: MSG: Mandibular salivary gland, MB: Mandible bone, PSG: Parotid salivary gland.



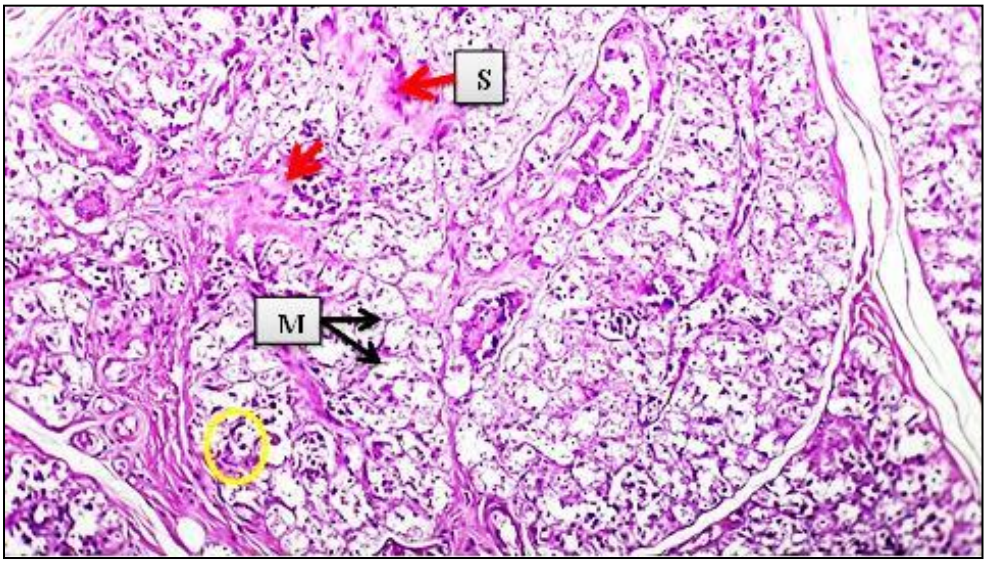


Figure (3) Histological structures of mandibular salivary gland in sheep show: M: Mucus acini, S: Serous acini, Yellow circle: serous demilune. H&E. 10X.

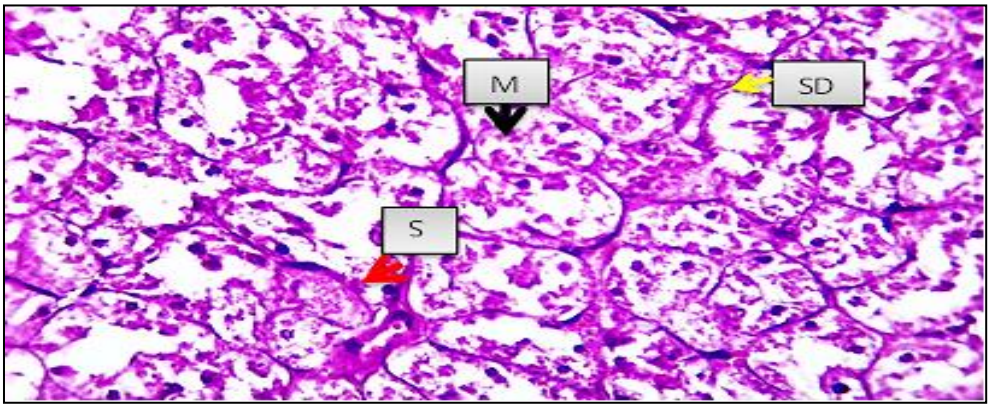
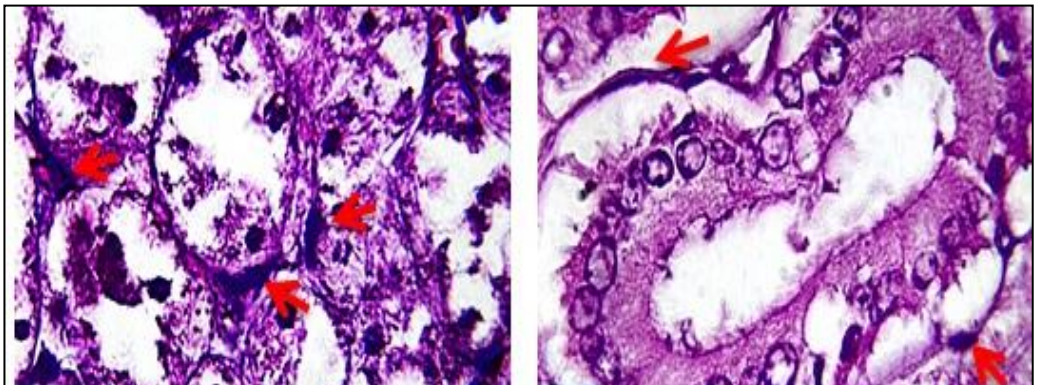


Figure (4) Histological structures of mandibular salivary gland in sheep show: M: Mucus acini. S: Serous acini. SD: Serous demine. H&E. 40X.



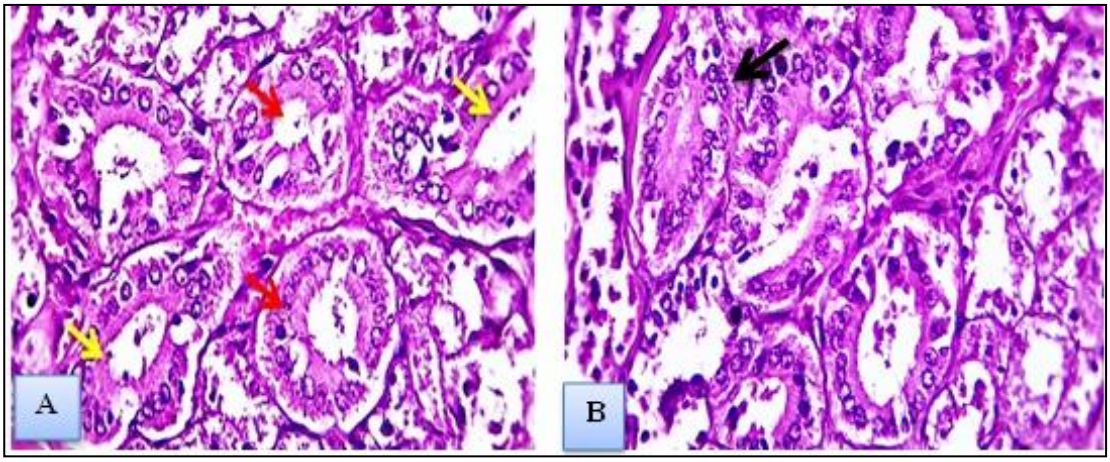


Figure (6)Histological structures of mandibulsr salivary gland in sheep show: A- Intercalate duct (red arrow) and Striated duc(Yellow arrows) . B- interlobular duct. H&E . 40X.

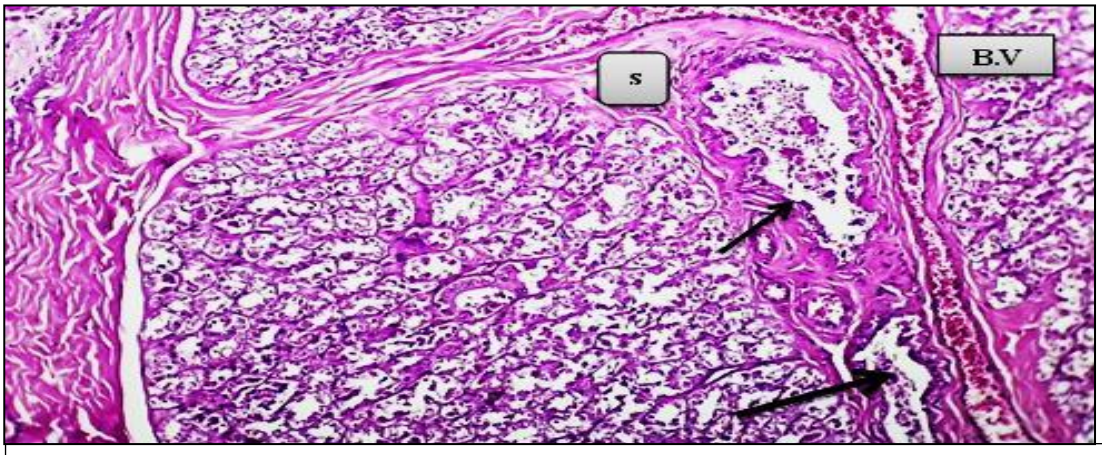
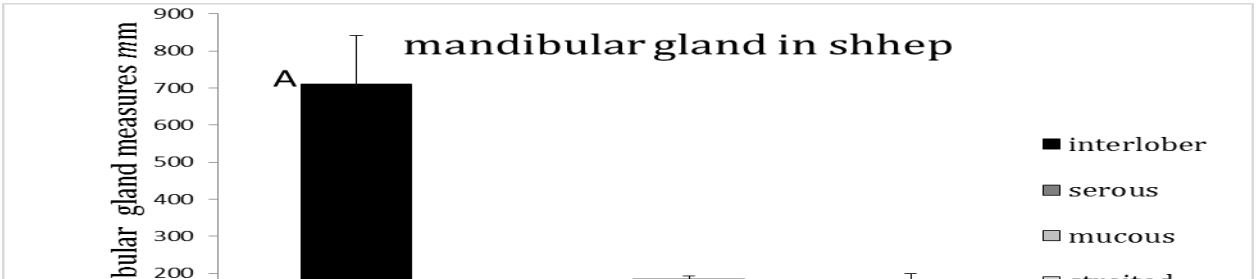


Figure 7: Histological structures of mandibular salivary gland in sheep show: A- Intelobar duct (black arrow) within septa (S), B.V. Blood vessels . H&E . 10X.



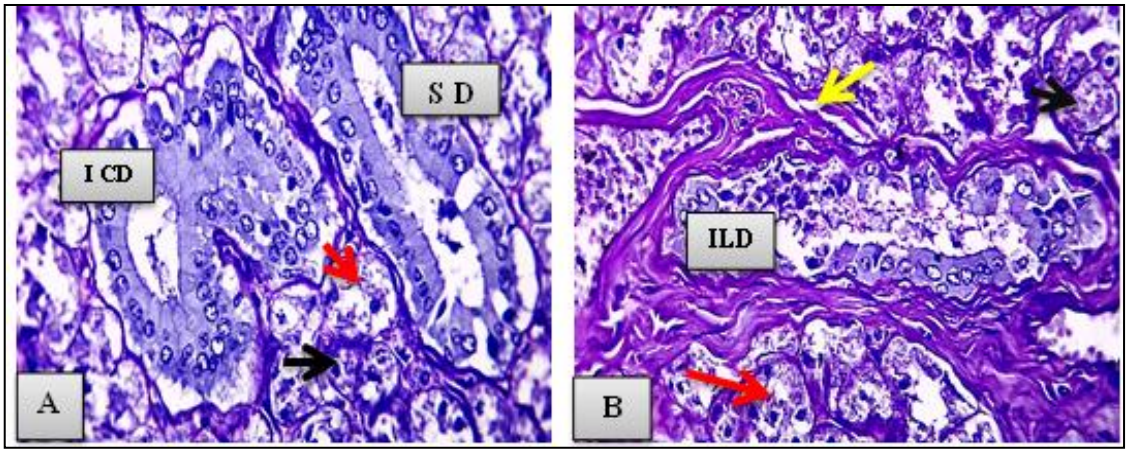


Figure 9. Histological structures of mandibular salivary gland in sheep show PAS stain reaction ICD: Intercalated duct, SD: Striated duct , Mucus acini (red arrow), Serous acini (Black arrow), ILD: Interlobar duct , Connective tissue (Yellow Arrow). PAS stain . 40X.

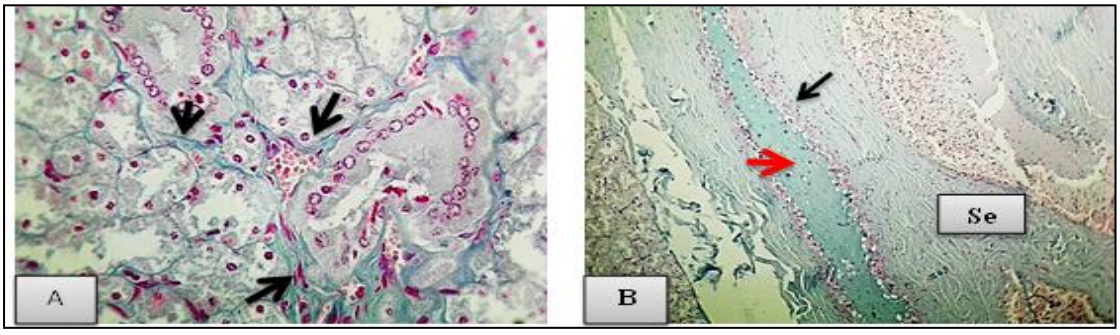


Figure 10. Histological structures of mandibular salivary gland in sheep show positive reaction to Alcian blue A- Around the mucous and serous acini (black arrows), B- Interlonular duct within the septa (Se), Secretion(Red arrow) . Alcian blue A. 40X, B- 10X

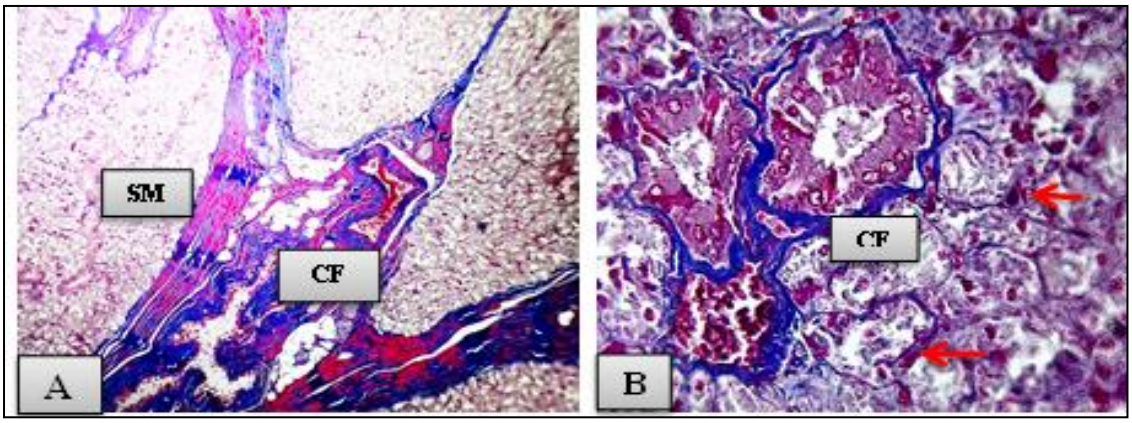


Figure 11. Histological structures of mandibular salivary gland in sheep show: CF: Collagen fibers, SM: Smooth muscle, Myoepithelial cells (red arrow). Alcian blue A. 4X. B- 40X Masson's Trichrom stain.

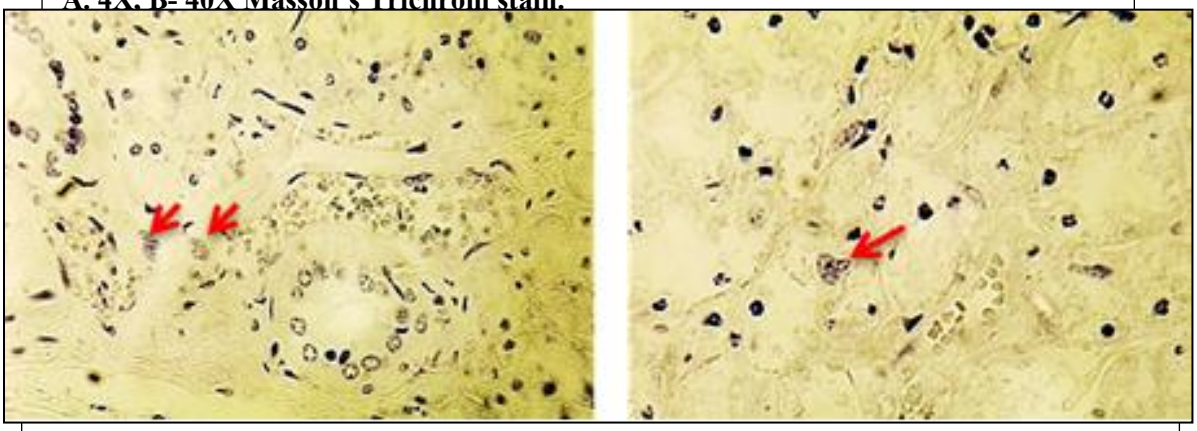


Figure 12. Weak positive staining of macrophage (Red arrows) in the mandibular salivary gland in sheep with CD 68PG M1antibodies. 40X.

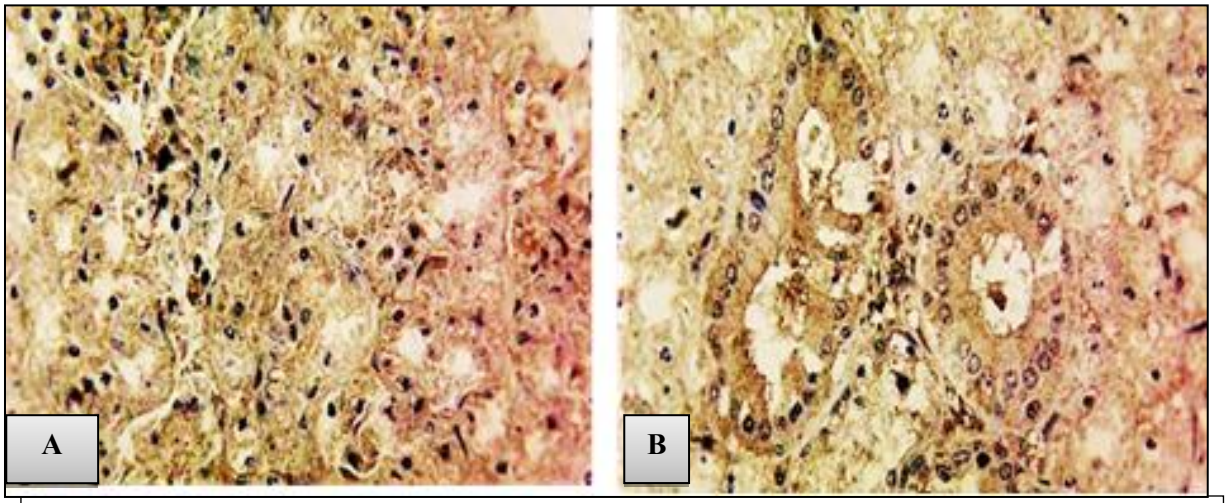


Figure 13. Positive staining of alkaline phosphatase activity in the mandibular salivary gland in sheep in: A-Moderate staining in the mucous and serous acini(light brrow), B-Strong staining in the striated duct and less in intercalated duct. Alkaline phosphatase 4X.

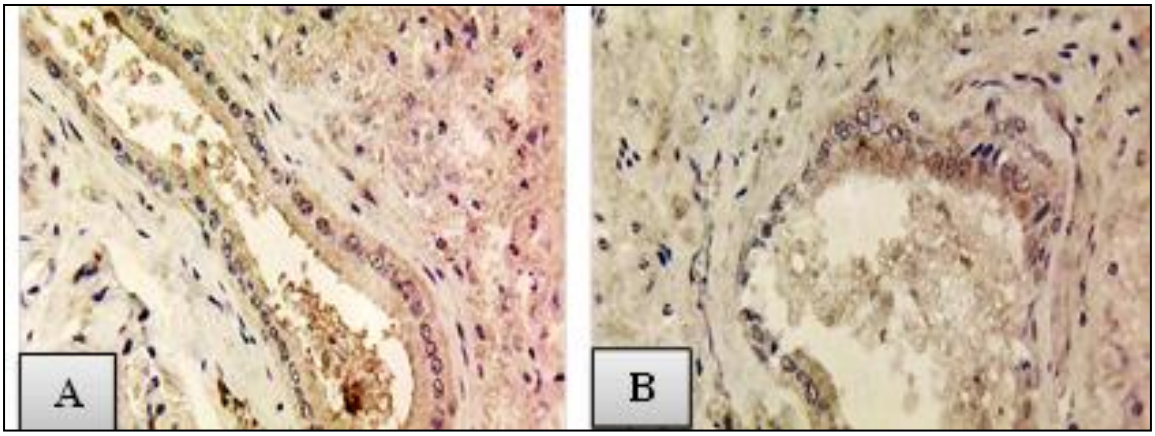


Figure 14. Positive staining of alkaline phosphatase activity in the mandibular salivary gland in sheep in: A-Moderate staining in the inter lobular duct (light brrow), B-weak staining in the striated duct and less in interlobar duct. Alkaline phosphatase 40X.