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RESEARCH ARTICLE

Histomorphological and histochemical study of the major salivary glands of adult local rabbits

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Abstract

The study aim to investigate histomorphological and histochemical structures of three major salivary glands in the adult local rabbits, that are parotid, submandibular and sublingual glands. To conduct that, twenty four adult local rabbits of both sexes are used. Macroscopically, all of the major salivary glands were paired and that the parotid was the largest compared to the submandibular and sublingual glands and the latter, was the smallest of these gland. Microscopically, parotid showed purely serous acini only in its constituting glandular tissue. Whereas, the other glands were mixed in which the submandibular was formed of mucous, serous and few demilune, but sublingual gland showed mainly mucous acini and many demilune only. The intralobular parenchymal tissue was very packed in case of submandibular gland, but for lower extent in parotid and for the lowest in sublingual gland. Characteristically, the microscopic sections from submandibular glands were showed higher number of intralobular ducts compared to those observed in the parotid and sublingual glands. Histochemical staining procedures were indicated various reactions of the glandular acini of these glands toward the different used stains in this study. Significantly, parotid acini were reacted positively with PAS stain, but did negatively with AB and combined AB-AF stains, because the gland was fully serous and no mucin could be detected in their acini. Similarly, submandibular showed PAS positive reaction. In addition to that the combined AB-PAS also showed positive reactions by the appearance of the blue and magenta colors to both stains, respectively. It indicated the presence of acidic and neutral mucins. Sections stained with AB showed dark blue staining indicated that their mucins of non sulfated type. Moreover, the combined AB-AF stains revealed positive reaction to AB but not the AF indicating the presence of acidic non sulfated mucin constituent in nature. Sublingual glands showed positive reaction with the PAS stain characterized by magenta color formation of its mucinous contents. In another hand, the combined AB-PAS stains also showed positive magenta color toward the PAS, but negatively reacted with the AB, indicated the absence of the non sulfated mucin. Whereas, AB-AF stains showed positive dark purple reaction due to AF stain, but not reacted with the AB part of the stain. Such findings indicated the presence of sulfated mucin in their constitutional acini.

Introduction

Rabbits are small mammals in the family Leporidae which is includes eight different genera, live in several parts of the world. Rabbits along with pikas and hares, make up the order Lagommorpha (Ghoshal and Bal, 1989). Generally,

experiments are conducted variability on laboratory animals provided at varying extent the knowledge which can be applied to humans and different domestic animals in veterinary practice (Mazensky et al., 2009). Actually, laboratory animals have been a direct object of a huge number of experiments that cannot be carried out in vitro. Rabbits are greatly selected nowadays to conduct various experiments and researches. Statistics reports of the United States Departments of Agriculture (USDA), showed that roughly 240000 rabbits used yearly to conduct researches within the U.S. (USDA, 2006). In fact, the advantages of these animals are that they are easily affordable and easy to control.

Currently rabbits are considered good experimental animal model in clinico-anatomical research of different morphological anomalies and particular diseases observed in both human and animals. They have been used as an experimental model in several experimentally induced diseases and for the aim to study of toxicology, pharmacology and surgery at many universities (Abidu-Figueiredo et al., 2008). Accordingly, microscopic structure of many organs such as the salivary glands in this animal is in need for depth description and investigation.

Salivary glands are important in researches because of their diverse functions (Asari et al., 2000). They are developed at different locations and they have very different architectures and manufacture different types of saliva (Jaskoll et al., 2002). The main salivary glands are paired parotid, submandibular and sublingual glands (Kimura et al., 1998). These glands play key role in the digestion of food with the aid of their secretion (saliva) which is frequently serous, containing diverse enzymes, water, mucopolysaccharides and lubricating glycoprotein (Adnyane et al., 2010). Therefore, the morphology, histochemistry and ultrastructure of these glands in different animal species have been the subject of numerous studies (Estecondo et al., 2005).

In general salivary glands are derived from epithelial cells and consist of parenchyma comprising the secretory units with their associated ducts and stroma which is the surrounding connective tissue that penetrates and divides the gland into lobules. The secretory units or the acini itself can be divided into three main types, that are serous, mucous and mixed types. The serous acinar cells are pyramidal in shape, with basally located nuclei surrounded by dense cytoplasm and secretory granules in the apex. Mucinous acinar cells are commonly simple columnar cells with flattened, basally situated nuclei and water-soluble granules that make the intracellular cytoplasm appear clear. Mixed, or seromucous, acini contain components of both types, but one type of secretory unit may dominate. Mixed secretory units are frequently observed as serous demilunes capping the mucinous acini. The acini first secrete through small canaliculi into the intercalated ducts, which in turn empty into striated ducts within the glandular lobule. The latter duct empty into interlobular excretory duct which is lined by pseudostratified columnar epithelium and embedded in dense fibrous connective tissue. Between the epithelial cells and basal lamina of the acinus, flat myoepithelial cells form a latticework and possess cytoplasmic filaments on their basal side to aid in contraction, and thus forced secretion of the acinus (Holsinger and Bui, 2007).

Histochemically, mucosal units of the salivary glands react strongly with staining techniques such as Alcian blue (AB) and PAS (Shackelford and Schneyer, 1964). Mucin of these glands possess acidic properties which are of two categories: acidic mucin and sulphomucins and when the mucin lacking delectable acidic properties it will be named as natural mucin (Kimura et al., 1998).

Developmentally, the submandibular is the first major salivary gland to be developed in the growing embryo, followed by the neighboring sublingual gland and subsequently by the parotid gland (Tucker, 2007).

The importance of salivary glands is their imperative roles in secreting many important digestive enzymes. Therefore, disorders that afflict these glands which can occur at both exocrine acini and their duct system may disturbs this function. So that research into their histomorphological and histochemical structures provides much insight into the etiology of such disorders, tumors and other diseases (Lebenthal, 1987). Tumors of salivary glands are uncommon in domestic animals; however, they have been reported in many species such as dogs, cats, cattle, sheep, horses, guinea pigs and goats (Head, 1976; Carberry et al., 1988). Recently, first report of salivary gland adenocarcinoma is recorded in the rabbit (Bercier et al., 2013).

In contrast to the huge number of studies conducted on the salivary glands of human, lack of studies on their histomorphological and histochemical structures in the local rabbit. Most investigations were carried out on the

laboratory animals such as rats (Watanabe et al., 1996), mice (Liu et al., 2000), ferrets (Triantafyllou et al., 2005) and hamsters (Yazadni Moghaddam et al., 2009), For a lesser extent, in the veterinary practice few studies were carried on the salivary glands of sheep (Dehghani et al., 2000), dogs (Asari et al., 2000), goat (Tadjalli et al., 2002), horses (Dehghani et al., 2005), cats (Kim et al., 2008) and cattle (Al-Sadi, 2013).

To our knowledge, there is no study documenting the histomorphological and histochemical features of the major salivary glands (parotid, sublingual and submandibular glands) in the local rabbits in Iraq, so as the present study intended to add knowledge on these glands in this important experimental animal model. The knowledge obtained could be constructing a basis for other veterinary or medical fields such as pathology and physiology. In view of the above, the present study was planned:

1. To study histomorphological structures of the major salivary glands that are parotid, submandibular and sublingual glands in the adult local rabbits.
2. To study Histochemically the acinar units and associated duct system of these glands

Materials and Methods

Twenty-four adult local rabbits (2.25–2.5 kg) were bought from the local suppliers at Baghdad province. Subsequently, they were kept under optimal hygienic conditions and had free access to commercially available pellet diet and tap water. Rabbits were submitted to two weeks acclimation period to minimize stress associated with transportation before their euthanasia by an intravenous administration of 150–200 mg/kg sodium pentobarbital (Euthasol; Delmarva Laboratories, Midlothian, VA) (Eifler et al., 2009).

Rabbits were divided into three groups: First group (n=8) are used for gross morphological approach to each investigated gland including shape, size and location. Glands were photographed in situ using digital camera. Weight of each rabbit is measured before its euthanasia. Fine dissection to the skin of the head is conducted to expose the major salivary glands. The size of each collected gland is measured in milliliters using water displacement method. Weight of each gland is measured in grams by using electronic sensitive balance.

Second group (n=8) are used to study the histological structures of the selected salivary glands in this study. After the animal euthanasia, the glands such as parotid, submandibular and sublingual glands are dissected free from the surrounding tissue, removed and fixed in 10% neutral buffered formalin.

Third group (n=16) were used to conduct histochemical study for each of the selected salivary glands in this study. Specimens of the glands are fixed in Bouin's solution. Specimens of both histological and histochemical study are dehydrated after their fixation in ascending series of ethyl alcohol (70%, 80%, 90% and 100%), cleared in xylene and embedded in paraffin. Six micrometer sections are prepared from the above specimens and then are stained with one of the bellow stains and subsequently examined and photographed by Olympus BH-2 microscope (Luna, 1968). The following general and histochemical stains were used as in the below:

General stains:

1. Harris hematoxylin & eosin stains for general morphological features.
2. Masson's Trichrome Method for the demonstration of the connective tissue fibers.
3. PAS stain for the carbohydrates, basement membrane.

Histochemical stains:

1. Alcian Blue (pH 2.5) for Acidic mucin.
2. PAS for neutral mucin.
3. AF for sulfated mucin.
4. AB-PAS for acidic & neutral mucins.
5. AF-AB for differentiate sulfated from non-sulfated mucins.

Results and Discussion

Gross morphology results and discussion of the parotid gland

The gross findings revealed that the paired parotid gland in the local rabbit is the largest gland compared to other major glands such as submandibular and sublingual. The gland is extended from the base of the external ear toward the angle of the mandible. It showed dorsal and ventral parts. It is surrounded by the fatty tissue characteristically in the female's specimens (Fig. 1). The gland extends below and in front of the base of the auricle of the ear between the skin and masseter muscle. Its duct is running rostrally along the lateral surface of the masseter muscle close to the branches of the facial nerve and finally enters the oral cavity opposite the last molar tooth. The color of the parotid gland is white or creamy and the lobulation of the gland was obvious with the naked eyes. The size and weight of the right parotid are slightly higher than those of the left parotid gland. The mean of their size is14 mm^3 at the right side, whereas it is 13mm^3 at the left side. The mean weight of the gland is 0.975 g ± 0.03SE, when the weights of rabbits used in the current study are ranged between 2.25 to 2.5 kg. The ratio of the gland weight mean to the body weight mean is 0.0043. The ratio appeared very low compared to previous measurement of the parotid gland in the minipigs in which the weight was 10.2 to 20.6 grams, equivalent to 0.3 to 0.7% of the animal's body weight (Wang et al., 1998). In another aspect, the ratio appeared higher than those recorded in the New Zealand rabbits in which the ratio of parotid to the body weight is about 0.00045 (Pratten et al., 1977).

The location of the parotid ventral to the base of the ear in the local rabbits is comparable to the location of parotid in other species such as rats (Kimura et al., 1998), rodents (Jonjic, 2001), Koala (Mizuno et al., 2009), dogs (Weidner et al., 2012) and human (Amano et al., 2012).

Parotid is paired and the largest major salivary glands in the local rabbit embedded in the subcutaneous adipose tissue, a character which is similar to what was observed in other species such as minipigs (Wang et al., 1998), New Zealand rabbits (Hakim et al., 2002) and in human (Holsinger and Bui, 2007). Whereas, in the mouse, the submandibular but not the parotid is documented as the largest major salivary gland in this laboratory animal species (Rosa et al., 2014).

Histological results and discussion of the parotid gland

The microscopic structure of the parotid gland in the local rabbits revealed that the gland is surrounded by very thin dense connective tissue capsule. Septae are extended from the capsule as well demarcated interlobular connective tissue with the presence characteristically adipose tissue separating the gland tissue into many lobules with different shapes and sizes (Fig. 2). In each lobule, very fine and scanty connective tissue found between the acini as intralobular septae, but the connective tissue is increased at areas surrounding the blood vessels and duct system such as the striated and larger interlobular ducts (Fig. 3).

Obviously, the gland showed prominently large area of the secretory units represented by the serous acini, parallel to scanty area including connective tissue, duct and blood vessels which are considered intralobular structures. Prominently, lobules are constructed of pure serous acini of small lumen and they are characterized by pyramidal-shaped lining cells with rounded basally located nuclei (Fig. 4). The intralobular duct system is initiated by the intercalated ducts which are lined by simple cuboidal epithelium, which in turn empties into larger striated ducts. The latter ducts are lined by simple columnar epithelium characterized by acidophilic cytoplasm and large basally located nuclei. These ducts are surrounded by fine connective tissue which are surrounded with many small blood vessels. The intralobular striated ducts are converged together to form the larger interlobular ducts which are lined by simple columnar epithelium. These ducts are mainly located in the septae which are constructed of wide extralobular fatty connective tissue. The large interlobular ducts are connected together to form the main excretory duct of the gland which is lined by pseudostratified columnar epithelium (Fig. 5) which is similar lining to the parotid duct in human (Fernandes et al., 2009).

The character of purely serous parotid gland currently observed in the local rabbit appeared similar to those previously observed in the parotid of other animal species such as the rat (Kim, 1976), rhesus monkeys (Stephens et al., 1986), mice (Al-Okaili, 2008) goat, sheep (Elewa et al., 2010), miniature pig (Zhou et al., 2010), barking deer (Adnyane et al., 2010), European hamster (Khojasteh and Delashoub, 2012), human and rodent (Amano et al., 2012) and African palm squirrel (Ekele et al., 2013). Whereas, different from those found in dog, cat, ferret (Poddar and Jacob, 1977) and Djungarian hamster (Suzuki et al., 1983) in which their parotids are mixed of serous and mucous acini. Recently, Elewa et al (2014) suggested that the serous type of parotid gland that observed in the herbivorous animals and the mixed one in the carnivorous could be related to the type of feeding in these animal species.

In fact, the wholly serous nature of the parotid gland of the local rabbit is similar to what was previously found in the New Zealand rabbits by Cope et al (1976). The latter, recognized higher percentage of acini forming the parotid tissue, whereas, the reminder lower percentage of the tissue is composed of ducts, fatty connective tissue, blood vessels and nerves.

Similarly to what was found in the local rabbit, the parotid gland tissue of Sprague Dawley rat showed many serous acini comprising extensive area of the gland tissue and a few ducts. But differently the interlobular connective tissue was not extensive in the rat which was fatty and obvious in the local rabbit (Miyazaki et al., 2008).

Current findings revealed the presence of myoepithelial cells surrounding the serous acini of the parotid in the local rabbit which is in consistence with the previous observations documented in the parotid of the Djungarian hamster in which the myoepithelial cells were detected around the acini constituting the gland tissue (Suzuki et al., 1983). Whereas, differently from rat parotid gland in which the serous acini lack myoepithelial cells and only cytoplasmic processes of myoepithelium of the intercalated duct could be found between their acini (Redman et al., 1980).

In the current histological findings, the intercalated ducts are connected to the secretory acinar units and from the other direction are connected to the intralobular striated ducts and such fact is dissimilar to those observed previously in some rodents such as the bat in which intercalated ducts are connected to the granular convoluted ducts and subsequently are connected to the striated ducts (Tandler et al., 1998). In fact, the granular convoluted ducts are absent in the parotid of the local rabbits which is a character detected in human (Harrison et al., 1987) and other animal species such as minipigs (Wang et al., 1998), ferret (Triantafyllou et al., 2005), Koala (Mizuno et al., 2009), barking deer (Muntiacus muntjak) (Adnyane et al., 2010) and African palm squirrel (Epixerus ebii) (Ekele et al., 2013).

The acini of parotid gland of the local rabbit are surrounded by myoepithelial cells, which are found around the intercalated and striated ducts too (Fig. 4). Whereas different character is observed previously in the dogs (Suzuki et al., 1975), horses (Suzuki and Otsuka, 1978) goats (Elewa et al., 2010) and African palm squirrel (Epixerus ebii) (Ekele et al., 2013), in which the myoepithelial cells are found only around the serous acini and the intercalated ducts of their parotid gland structure.

Histochemical results and discussion of the parotid gland

As it is mentioned in the above, the nature of parotid acini in the local rabbit are fully serous and that are mixed in some other species. Accordingly, variable histochemical reactions could be detected with different histochemical stain procedures.

Sections from the parotid gland of the local rabbit which are stained with PAS revealed positive reaction in their acinar cells as well as the associated intralobular striated ducts (Fig. 6). Whereas, there is no reaction with Alcian blue (pH 2.5) as well as combined AB (pH 2.5) –Aldehyde fuchsin stains could be detected. Such negative reaction with AB is also documented in the parotid glands of minipigs (Wang et al (1998) , Koala (Mizuno et al., 2009) goat, sheep and mouse (Elewa et al., 2010) because the gland is fully serous similar to the current studied local rabbit. The latter researcher observed moderate reaction with the PAS stain in the goats and sheep whereas, intense reaction in the mice parotid acini. Moreover, the parotid glands of both goat and sheep showed a few mucous secreting cells with intense positive reactions for both PAS and AB stains appeared in the distal portions of the interlobular ducts, but such positively reacting cells were not found in the mouse.

Previously, Suzuki et al (1983) detected similar histochemical reactions to those of local rabbits in the acinar cells of the parotid glands of Djungarian hamster. The parotid acini of this species is contained acidophilic granules which reacted positively with the PAS. But, dissimilarly to local rabbit, parotid acini of hamster slightly stained with Alcian blue (pH 2.5) because the gland is seromucous in nature so that their mucous acini are only reacted positively with this stain, whereas, in the local rabbit is negatively reacted with this stain.

Parotid glands of ferret showed different reaction from that of local rabbit toward the AB (pH 2.5) – PAS stain because their acinar cells are variably stained in shades of purple and royal blue with AB (pH 2.5) – PAS procedure.

Whereas, most acinar cells had an affinity for AB alone which correspond fully negative reaction in the local rabbit. However, only granules present in the lining epithelium of the striated ducts stained are with PAS (Triantafyllou et al., 2005). Comparing the current histochemical findings to those observed in the parotid glands of the adult barking deer (Muntiacus muntjak), revealed fully different reactions (Adnyane et al., 2010). The acini in this species showed complete negative reaction with both AB and PAS stains, because they have not acidic or neutral carbohydrates in their serous acini, respectively. Current findings revealed negative reactions with both AF and the combined AF-AB stains. The non stainable acini of rabbit parotid with these stains is an indication of their lack to the sulfated acidic and neutral mucin in their acinar cells.

Gross results and discussion of the submandibular gland

The gross findings revealed that the submandibular gland in the local rabbits is smaller in size compared to the parotid gland but in another hand its size is larger than those of the sublingual gland in this animal. The glands is characterized by pyramidal shape and red-brown in color. The external surface of the gland is smooth and no sign of gross lobulation could be observed. The latter character is different from that observed in the parotid of the same animal species in which its lobulation can be observed with naked eye. Both right and left submandibular glands are located medially to the angle of the mandible. The glands are situated juxtapose each other in the middle line at the caudal part of the tongue (Fig. 7). Similarly to the parotid gland, the right submandibular gland is larger than those of the left side. The mean size of the right gland is 5 mm^3, whereas, that of the left one is 4 mm^3. The mean weight of the gland is 0.430 g ± 0.23. The ratio of the submandibular glands/body weight means is 0.0064. The mean weight of the gland appeared lighter compared to that of the parotid. The weight of the gland in the local rabbit is less than those reported for the Sprague Dawley rat which was 0.79± 0.079 g when the body weight of the rat was 223.6 ± 9.52 g (Haghighat and Hashimi, 1999). Whereas, the weight was higher than those reported in the Wistar rat submandibular gland which was 0.208g when the rat weight was 250-350 g (Cotroneo et al., 2010). The weight of the submandibular gland in the minipigs is 8.0 to 11.5 g which is corresponded to 0.026% to 0.039% of the animal's body weight, indicating higher ratio compared to those of the local rabbit (Xin et al., 2005).

Differently from the local rabbit, the submandibular glands in the mouse is the largest and is multilobed and lobulated (Rosa et al., 2014), whereas, it is smaller than the parotid and in another aspect larger than the major sublingual glands in the rabbit, in addition to that the gland is just single lobe and not multilobed.

The location of the submandibular gland in local rabbit is similar in its position to the same gland in the human as they juxtapose each other in the middle line caudal to the base of the tongue (Hakim et al., 2002). In rodents, Yazadni Moghaddam et al (2009) also documented that the submandibular glands are located in the posterior end of the tongue.

The submandibular gland in local rabbit is situated caudal to the sublingual gland and each of them has its own surrounding capsule and such position was similarly found in the hoary bamboo rat but differently both glands are enclosed with same capsule (Kimura et al., 1998). As same as to rodent, in dogs both submandibular and sublingual glands are enclosed within a strong fibrous capsule (Weidner et al., 2012).

The major ducts of the submandibular gland are situated and ended on either side of a midline in the floor of the mouth. The duct is well exposed when the tongue is displaced to one side and the floor mucosa is dissected for a short distance. Similar findings regarding the end opening of the main duct of the parotid is also documented previously in the New Zealand rabbit by Bhaskar et al (1966).

Histological results and discussion of the submandibular gland

The submandibular gland of the local rabbit is surrounded by dense regular connective tissue capsule (Fig. 8). Fine interlobular connective tissue septae are extended from the capsule dividing the gland tissue into lobules with different shapes and sizes. Differently from parotid, the interlobular septae lack the adipose tissue which was prominently observed in that gland (Fig. 9). Each lobule is composed of densely packed acini. The gland is mixed in nature in which their acini are mainly mucous and for a restricted extent of serous types which constitutes the major bulk of the lobules. There are few mucous acini capped by serous demilune (Fig. 10). Serous acini are smaller in size than the mucous ones and their lumen are always very small and obscured, whereas, the mucous type are showed small but obvious lumen. The latter is lined by pyramidal cells possessed oval nuclei rested on the base of the cell.

While in serous acini, the nuclei are larger and rounded in shape, situated in the basal half of the cells. Myoepithelial cells are detected around the acinar units (Fig. 11).

The intralobular ducts such as intercalated and striated ducts are intervening between the acini which are lined by simple cuboidal and columnar epithelium, respectively (Fig.12). The latter is surrounded by myoepithelial cells but not the intercalated. Characteristic histological trait of the gland is the presence markedly considerable and higher number of intralobular ducts in the histological sections compared to those of parotid and sublingual glands (Fig. 9). The simple cuboidal and simple columnar lining epithelium of the intercalated and striated ducts in the local rabbit is comparable to those found in the submandibular glands other species such as adult baboon, Rhesus monkeys **(**Boshell and Wilborn, 1983), ferret (Triantafyllou, 1999), European hamster (Khojasteh and Delashoub, 2012) and African giant rat (Ikpegbu et al., 2013).

Extremely very thin interstitial tissue are difficulty detected between the acini. However, sections stained by Masson Trichrome, the collagen fibers could be recognized well around the blood vessels and large intralobular ducts (Fig. 13). The interlobular connective tissue septae that are separated different lobules revealed the presence of interlobular and the excretory ducts. The smaller ducts are lined by a simple columnar epithelium, whereas, the larger ducts are lined by pseudostratified columnar epithelium (Fig. 14) and such lining is also observed previously in the main excretory duct of the mouse submandibular gland (Sato et al., 1992).

The gland in the local rabbit is found mixed in nature in which their mucous acini are more numerous than the serous ones and such character is also reported in other species such as the submandibular gland of the rhesus monkeys (Stephens et al., 1986), cat (Sozmen et al., 1999), tree shrew (Zainuddin et al., 2000), Wistar rats (Coire et al., 2003), minipigs (Xin et al., 2005), bovine (Adnyane et al., 2007), barking deer (Adnyane et al., 2010) and European hamster (Khojasteh and Delashoub, 2012). Recently, findings of EL-Kordy et al (2014) showed similar picture to the local rabbit's submandibular salivary gland in the cat. He found mucous and serous secretary end pieces often capped with the myoepithelial cells are present between the base of the acinar cells and the basal lamina

Similar to the local rabbit, in the human the majority of acini of submandibular gland are of mucous type and the remainder contains mucous cells capped by serous demilunes but no serous acini is observed as it is found in the local rabbit' submandibular gland (Harrison et al., 1987). In the dog (Pedini et al., 1994) and goat (Habata et al., 2012) different observations are documented to those of local rabbit but similar to those of human by the presence of mucous and demilune acini only in their submandibular gland. Whereas, in the Koala, the submandibular is totally different from those of the local rabbit by having serous acini only in their structure (Mizuno et al., 2009).

The parenchyma of the submandibular gland in the ferret is different from that of the local rabbit because their lobules comprised mixed acini of palely stained mucous central cells that are often capped by small and densely stained demilunes and complete absence of the serous acini in their structure (Triantafyllou et al., 1999).

Totally different to the local rabbit, Ikpegbu et al (2013) mentioned that the histological structure of the submandibular gland in the African giant pouched rat is constructed from two distinct regions which are clearly visible, one of them contained mostly serous cells while the other contained mostly mucous cells and that some mucous cells are capped by serous demilune. Approximately similar differences as in the above observed previously in the histological structure of the submandibular gland of the mice compared to the local rabbit. Both mucous acini and serous acini are found but these two types of acini organized as separated groups, each of its own type (Al-Okaili et al., 2008). However, in the Jaculus blanfordi which is a species of rodent in the Dipodidae family contains only serous acini. It is hypothesized that the differences in submandibular glands of rodents might be associated with differences in their diet. Moreover, the diversity in the histological structure of the submandibular gland maybe originates from the differences of their living environments (Yazadni Moghaddam et al, 2010).

Three different types of ducts are present in the submandibular of the local rabbit that are intercalated, striated (intralobular) and excretory ducts (interlobular) same as found in other species such as human (Harrison et al., 1987), ferret (Triantafyllou et al., 1999), minipigs (Xin et al., 2005), barking deer (Adnyane et al., 2010) and European hamster (Khojasteh and Delashoub, 2012). Whereas, the ductal system of some other species is different structurally from that of the rabbit by consisting of four distinct segments, that are intercalary duct, granular duct, striated duct

and excretory duct, as in the gerbil (Bazan et al., 2001), rodents (Yazadni Moghaddam et al., 2009) and rat (Tsuboi et al., 2010) submandibular glands.

The presence of myoepithelial surrounding the acini in submandibular salivary gland of the local rabbit appeared similar to those observed in the acini of the same gland in human, rat (Ogawa, 2003), European hamster (Khojasteh and Delashoub, 2012), African giant pouched rat (Ikpegbu et al., 2013) and cat (EL-Kordy et al., 2014). The lack of myoepithelial around intercalated duct of local rabbit is similar to what was found in rat submandibular (Brocco and Tamarin, 1979; Ogawa, 2003) but differently, Ikpegbu et al (2013) observed these cells surrounding intercalated ducts in the submandibular salivary gland African giant pouched rat.

Histochemical results and discussion of the submandibular gland

Sections of submandibular gland of the local rabbit which are stained with PAS showed many acini with magenta color indicated positive reaction to their neutral and acidic mucin (Fig. 12). Combined AB (pH 2.5) -PAS revealed many acini with blue and magenta colorations indicating positive reactions to AB and PAS, respectively (Fig. 14). Whereas, Alcian blue (AB) (pH 2.5) stained sections are reacted positively (dark blue) with the mucous acini indicating that their mucous is acidic and non sulfated mucin type (Fig. 15). Alcian blue – Aldehyde fuchsin (AB-AF) stained section of submandibular gland showed positive reaction (deep blue) to AB stain but negative reaction to the AF because mucous acini of the gland is of acidic and non sulfated type and the latter stain strongly with the sulfated type only (Fig. 16). The type of mucous in the local rabbit is different from that reported previously in the cat's submandibular gland in which the mucous acini are positively reacted to AF because they possess sulfated mucous secretory materials (Sozmen et al., 1999).

The results of the combined AB-PAS in the local rabbit's submandibular gland is in a good agreement with those observed in the submandibular glands of hoary bamboo rats (Kimura et al.,1998) and goats (Habata et al., 2012) in which the sections stained with AB (pH 2.5) – PAS moderately (magenta) with the serous demilunes, while strongly (bluish magenta) with the mucous cells.

The positive reactions of the combined AB (pH 2.5) -PAS in the local rabbit's submandibular gland is parallel to those observed previously in the same gland in minipigs (Xin et al., 2005) and goats (Habata et al., 2012). In fact, these animal species consists of mixed submandibular gland, in which their mucous type are reacted strongly similar to the local rabbit's submandibular with the Alcian blue and periodic acid Schiff. Whereas variable observations recently recorded in the Barking deer's submandibular gland (Adnyane et al., 2010) in which the serous acini showed negative reaction toward combined AB and PAS stains and their mucous acini showed variable positive reaction.

Gross results and discussion of the sublingual gland

The gross findings revealed that the sublingual gland have the smallest size compared to those of parotid gland and submandibular glands in the local rabbits. It is located rostrally for a distance to the submandibular gland at the ventrocaudal aspect of the tongue close to the root. The glands characterized by its elongated shape and is red-brown in color. Its external surface is smooth and no sign of gross lobulation could be observed (Fig. 17). Both right and left sublingual glands have the same size. The mean size of the right and left gland was 1.25 mm^3. The mean weight of the gland was 0.145 g ± 0.18. The ratio of the sublingual glands/body weight means was 0.00064.

Similar to the local rabbit, the sublingual glands in humans are found to be the smallest of the major salivary glands and also located deeply under the mucous membrane of the floor of the mouth (Rana et al., 2012). But in another aspect, the connective tissue covering the gland is very thin and almost transparent, so that the lobulation could be seen through, whereas the lobulation is not observable grossly in the local rabbit.

Currently, the location of the rabbit's sublingual gland is rostrally for a distance to the submandibular gland, whereas in rodents is located together with the submandibular glands in the anterior neck spaces between the submandibular lymph nodes and the sternum and both of them are encapsulated with a common fascia **(**Amano et al., 2012). Similar to the rodents but not to the local rabbits, the major sublingual gland of dogs is invested within a strong fibrous capsule fused with mandibular gland, thus its delineation is difficult (Weidner et al., 2012).

Whereas, likewise to the local rabbits, the mouse sublingual gland is also a single lobe and appeared smaller than the other major salivary glands of the same animal (Rosa et al., 2014).

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Histological results and discussion of the sublingual gland

Microscopically all of the glands is surrounded by a very thin connective tissue layer forming the capsule of variable thickness from which septa go inside and divides the glands into several lobules with different sizes and shapes. Adipose tissue is well intervening inside the capsule and interlobular septae (Fig. 18). Delicate and very fine connective tissue intralobular septae are present between the acini which are difficult to be observed even with special stain such as Masson Trichrome (Fig. 19).

The gland is consisted of mixed acini. Lobules constructed mainly of mucous acini and for a lesser extent of demilune and the intralobular ducts such as intercalated and striated ducts. The gland histological structure is different from that of the submandibular gland of the same animal by lacking to serous acini and the presence of capped mucous acini by basophilic cells, as crescent shape demilunes. Demilunes are prominent and their number is marked compare to their few number in the submandibular. Well defined and visible lumina are observed in the mucous acini which are lined with eosinophilic pyramidal-shaped cells surrounded by myoepithelial cells which are detected around the associated striated ducts too. Intercalated and striated ducts are lined with simple cuboidal and simple columnar epithelium, respectively. Very rich blood vessels are associated with the intralobular ducts which are intervening between the acini. The number of intercalated ducts is very few compared to those of the striated ducts. The interlobular ducts are lined with simple columnar epithelium which are converge to form the main excretory duct which is lined by pseudostratified columnar epithelium (Fig. 20, 21).

The sublingual gland structure of the local rabbit is different histologically from that of the human. Because in human, it showed mucous, serous and demilune in their sublingual glands (Rana et al., 2012). The latter, found few number of striated ducts in human glands, whereas, differently the intercalated ducts are few in number in the local rabbits.

Previous findings of McLaughlin et al (1990) revealed that the sublingual gland in the rabbit is almost entirely mucus producing gland indicating that their acini are mainly mucous in nature and such findings are confirmed currently by the presence mainly mucous acini beside low number of serous demilunes in the structure of local rabbit's sublingual gland. Thus, the higher or paucity number of the striated ducts in the sublingual gland explain the different tonicity of the saliva liberated by these glands (Chauncey et al., 1966 in Rana et al., 2012).

The presence of myoepithelial cell surrounding the acini and striated ducts but not the intercalated duct of the local rabbit's sublingual glands is similar to what is found previously in the sublingual gland of the monotreme echnida (Raubenheimer et al., 1987).

Histochemical results and discussion of the sublingual gland

Microscopic examination of sections stained with PAS stain revealed positive reaction with this stain characterized by faint magenta color of the mucous acini of the sublingual gland of the local rabbit. The reaction indicated the presence of acidic mucin in their acini. Positive reaction also observed in the intralobular and interlobular ducts (Fig. 21) as well as the main excretory duct.

The sections which are stained with the combined AB-AF stain showed positive reaction (dark purple) toward the AF part of the stain indicated that the sublingual mucous acini of the local rabbit are sulfated acidic mucin in nature. Whereas, AB part of the stain is negatively reacted with the mucin of the mucous acini, because the stain is well reacted in case of non sulfated mucin only (Fig. 22). The sulphated mucous type of the local rabbit's sublingual gland is in agreement with remarks of Sozmen et al (1999) whom mentioned that the sulfated mucous is predominate in the sublingual glands of the cats.

The combined AB (pH 2.5) -PAS stained section of the sublingual gland revealed positive reaction (magenta color) toward PAS, whereas, negative reaction toward the AB one, indicating the absence of non sulfated acidic mucin in their mucous acini. Alcian blue stain alone, showed negative reaction in their mucous acini but not the striated duct which are stained deep with the stain. Whereas, the case is different in Koala (Mizuno et al., 2009) and Malayan pangolin (Manis javanica) (Munyala et al., 2008) sublingual's glands because their acini stained with both stains of

the combined AB-PAS The resulted reactions is due to the presence of mucous, and serous acini in their histological structure, whereas, in the local rabbit only the predominated mucous acini and the demilune and absence of the serous acini.

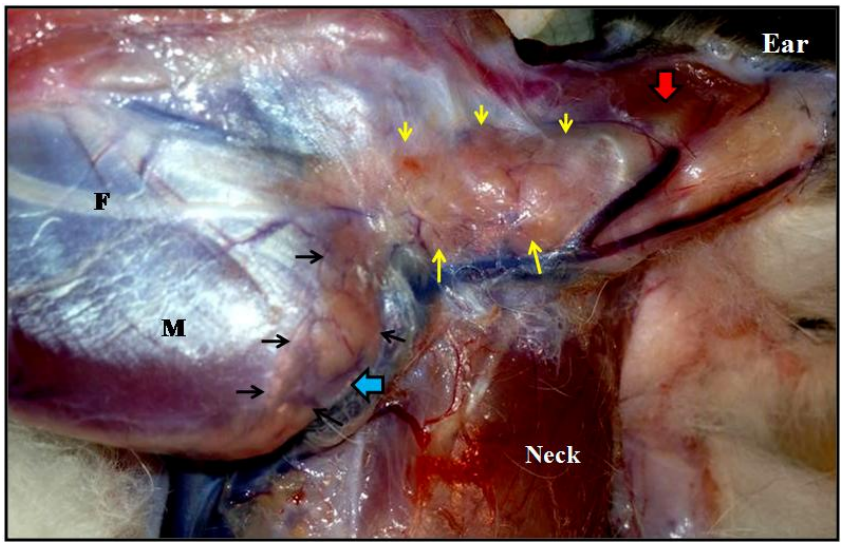

Fig.1. Gross appearance of the parotid gland in the local rabbit. It showed dorsal part (yellow arrows) located ventral to the base of the ear (red arrow) and the ventral part (black arrows) confined to the angle of the mandible (blue arrow) and covering the masseter muscle (M). The facial nerve (F) extended anteriorly approximately from the mid-area between the two parts of the gland.

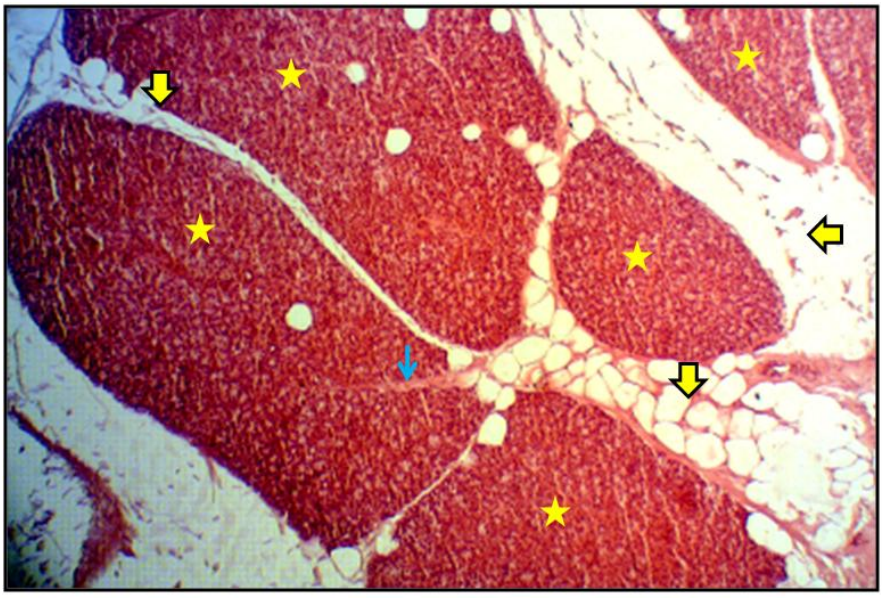

Fig. 2. Section from parotid gland of local rabbit. It showed many lobules of serous acini (yellow stars) separated by interlobular connective tissue septae with prominent appearance of the adipose tissue (yellow arrows) from which very fine intralobular septae are extended into the lobular tissue (blue arrow), H&E, X100

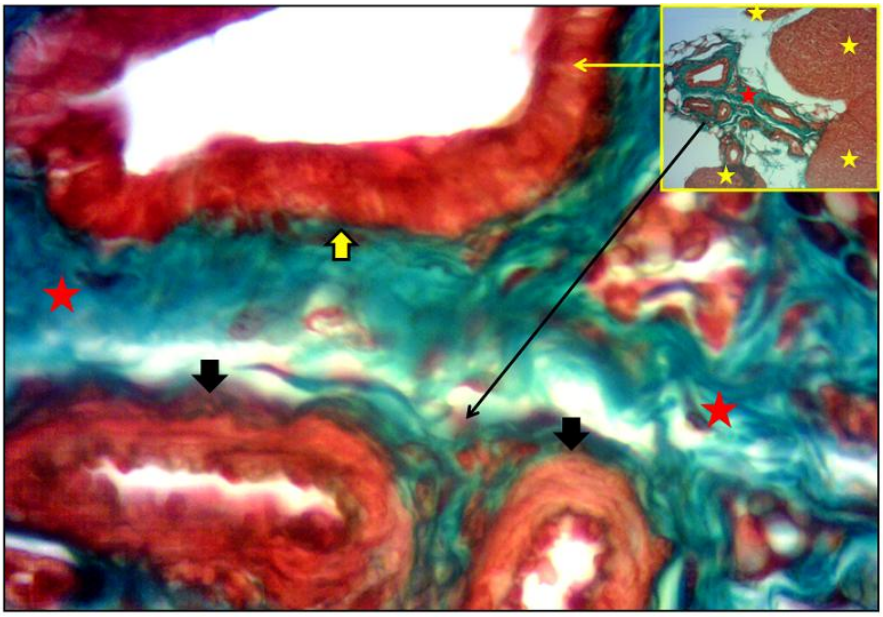

Fig. 3. Section from parotid gland of local rabbit showed interlobular region. The region showed many lobules constructed from serous acini (yellow stars). Thick interlobular connective tissue septae (red stars) surrounding large excretory duct (yellow arrow) and many muscular arteries (black arrows), Masson's Trichrome, X40 (yellow rectangle) and X400 (the main figure)

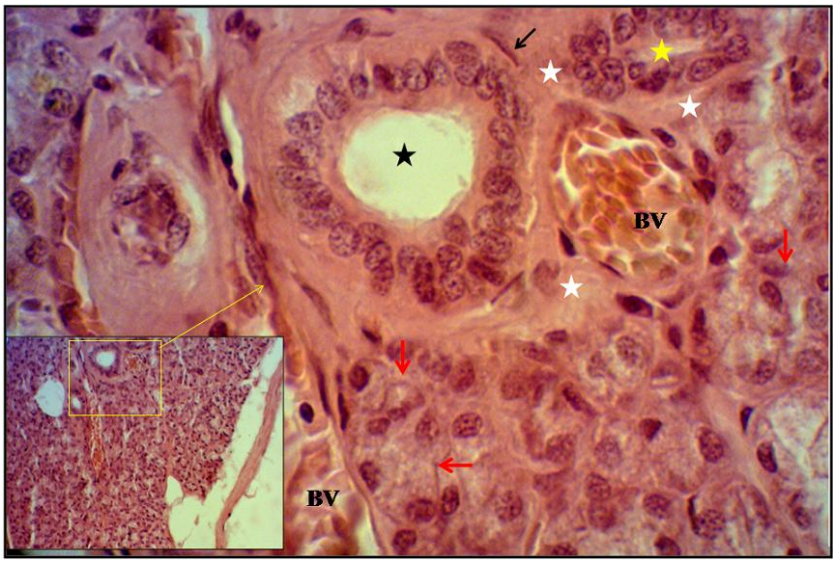

Fig. 4. Section from parotid gland of local rabbit showed intralobular region. The region showed serous acini (red arrows) characterized by small lumen and basally located rounded nuclei. Thick intralobular connective tissue (white stars) in which present intralobular ducts such as intercalated (yellow star) and striated (black star) and blood vessels (BV). Myoepithelial cell (black arrow) surrounds the striated duct, H&E, X40 (yellow rectangle and X400 (the main figure)

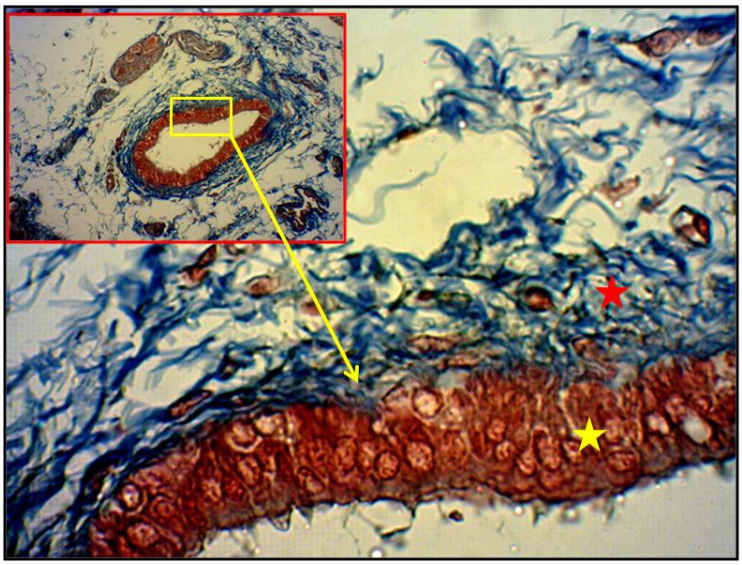

Fig. 5. Section from parotid gland of local rabbit showed large excretory duct. Interlobular region made of thick connective tissue (red star) surrounding the excretory duct which is lined with pseudostratified columnar epithelium (yellow star), Masson's Trichrome, X40 (red rectangle) and X400 (the main figure)

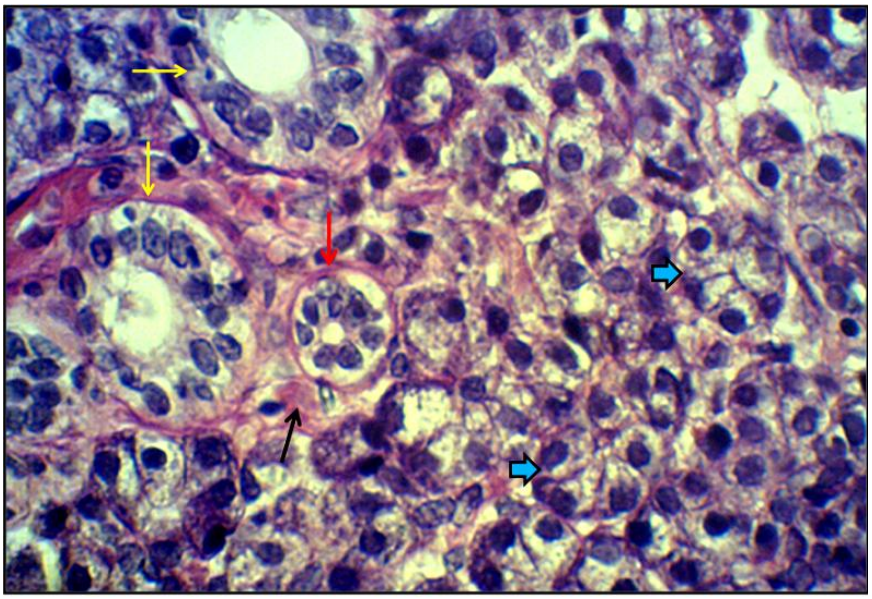

Fig. 6. PAS stained section of the parotid gland of local rabbit. It showed positive reaction (blue nuclei) of the serous acini (blue arrows) and the striated ducts (yellow arrows) to the PAS stain. Positive reaction (magenta color) of the surrounding septae (red stars), PAS, X40 (red rectangle) and X400 (the main figure)

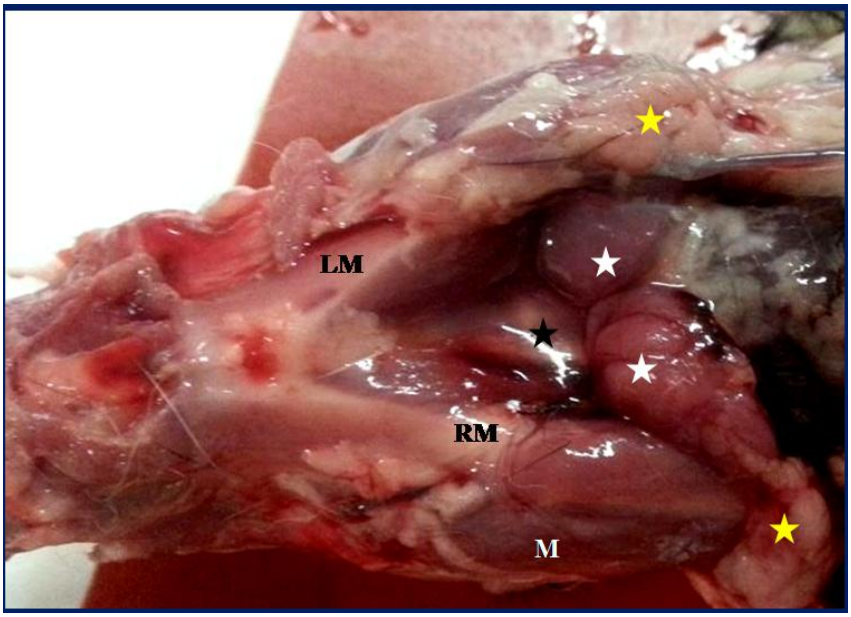

Fig. 7. Gross appearance of the submandibular gland in the local rabbit. It showed right and left glands (white stars) which are situated juxtapose each other in the middle line at the caudal part of the tongue (black star). The figure also showed part of parotid glands (yellow stars), masseter muscle (M) and left and right mandibles (LM, RM).

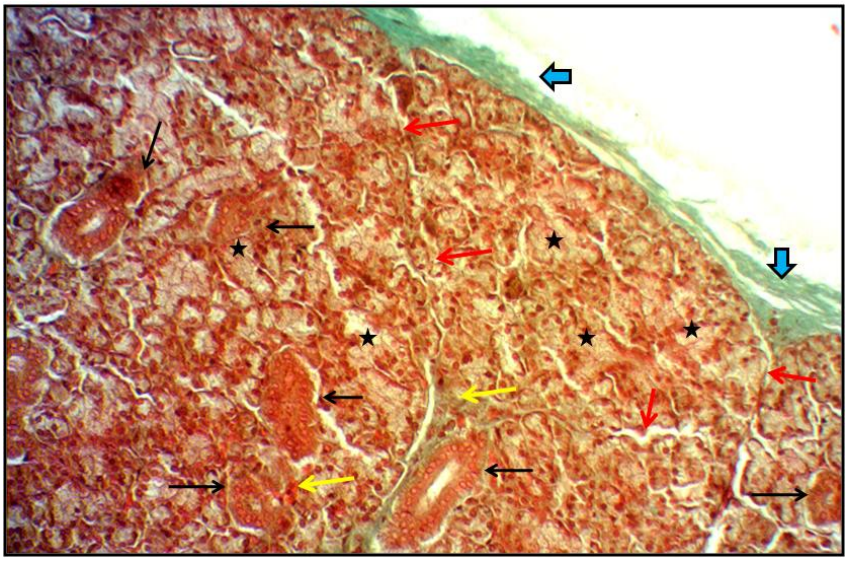

Fig. 8. Section from submandibular gland of local rabbit. It showed thin connective tissue capsule (blue arrows). The capsule sent very delicate septae (red arrows) between its mixed acini (yellow stars) which are thickened around the blood vessels (yellow arrows) and ducts (black arrows), Masson's Trichrome, X100

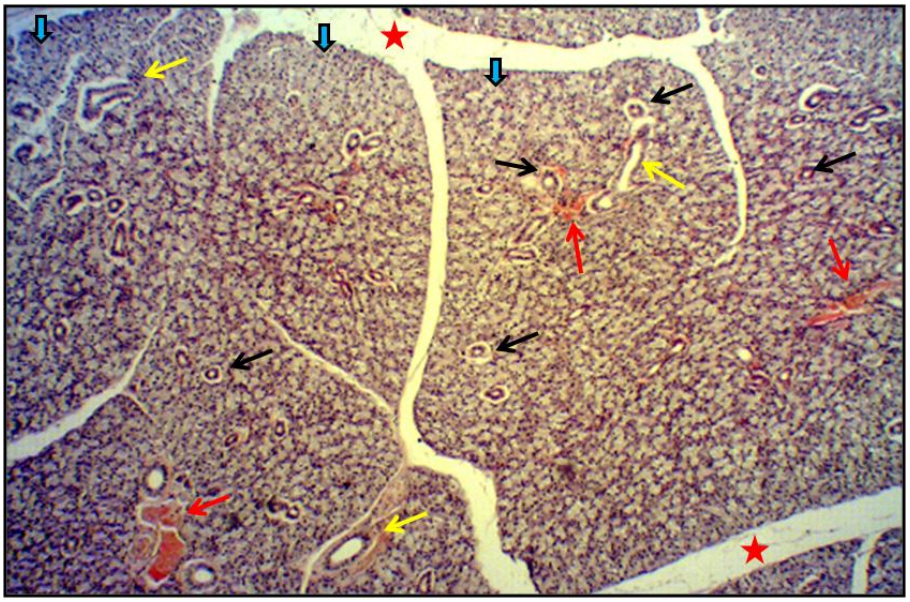

Fig. 9. Section from submandibular gland of local rabbit. It showed interlobular connective tissue septae (red stars) dividing the parenchyma into lobules which showed mixed acini (blue arrows) with their associated striated ducts (yellow arrows), intercalated ducts (black arrows) and intralobular blood vessels (red arrows), H&E, X40

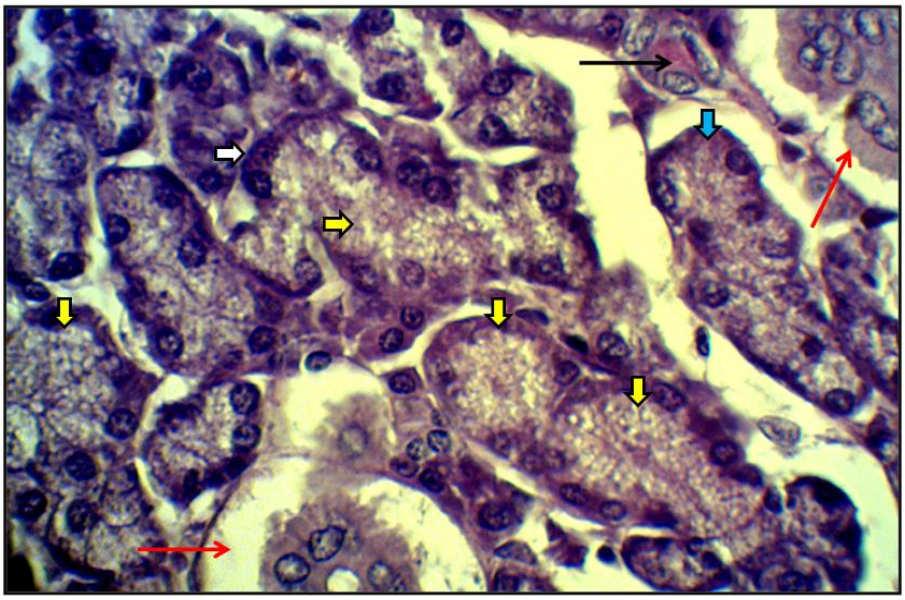

Fig. 10. PAS stained section from submandibular gland of local rabbit. It showed mucous acini (yellow arrows), serous acinus (blue arrow), serous demilune (white arrow) and intralobular intercalated (black arrow) and striated ducts (red arrows), PAS, X400

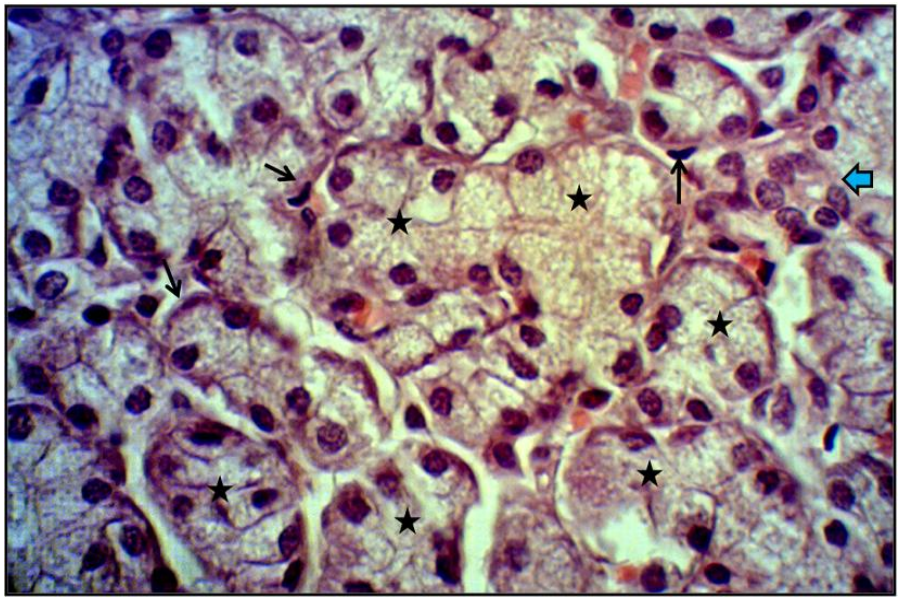

Fig. 11. Section from submandibular gland of local rabbit. It showed myoepithelial cells (black arrows) surrounding the acini (black stars), but not the intercalated duct (blue arrow), H&E, X400

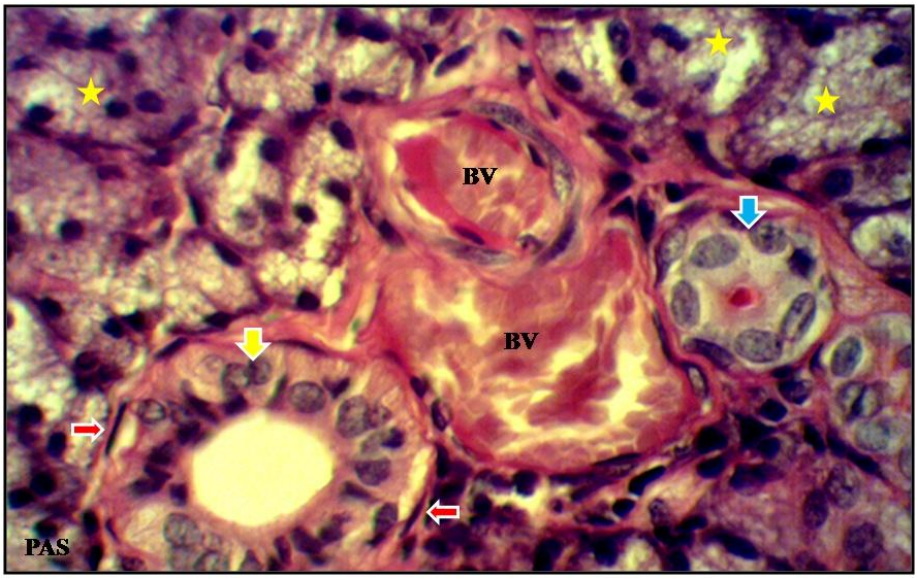

Fig. 12. PAS stained section from submandibular gland of local rabbit showed myoepithelial cells. The myoepithelial cells (red arrows) are present around the striated duct (yellow arrow) but not the intercalated duct (blue arrow). The figure showed many acini (yellow stars) with magenta color indicated positive reaction to neutral and acidic mucin and blood vessels (BV), PAS, X400

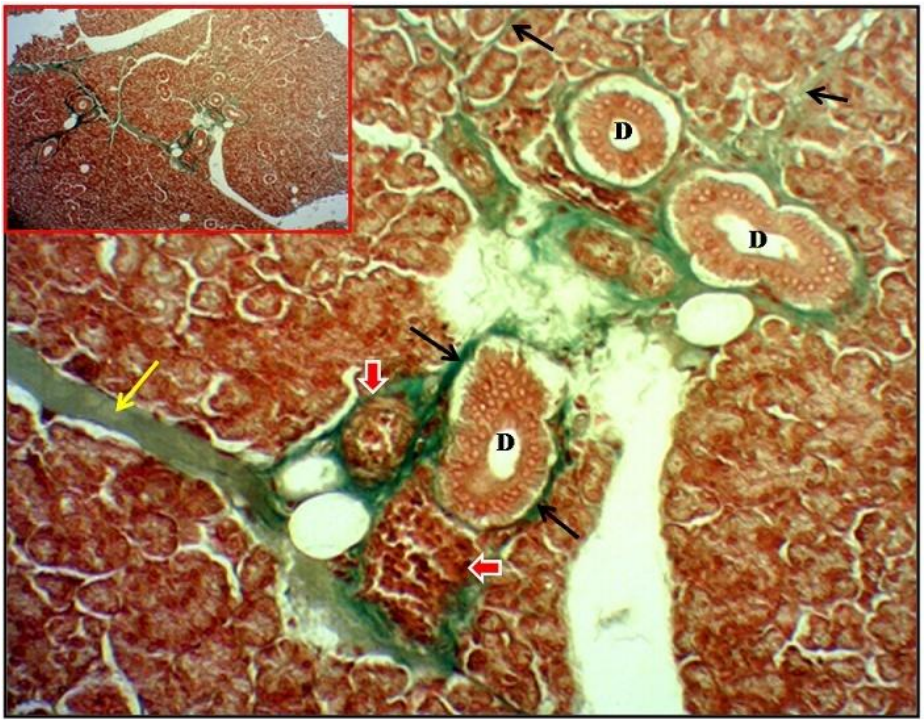

Fig. 13. Submandibular gland of local rabbit showed intralobular connective tissue septae. The figure showed prominent intralobular connective tissue surrounding the intralobular ducts (D) and blood vessels (red arrows) which is originated from the interlobular connective tissue septae (yellow arrow), Masson's Trichrome, X40 (red rectangle), X100 (the main figure)

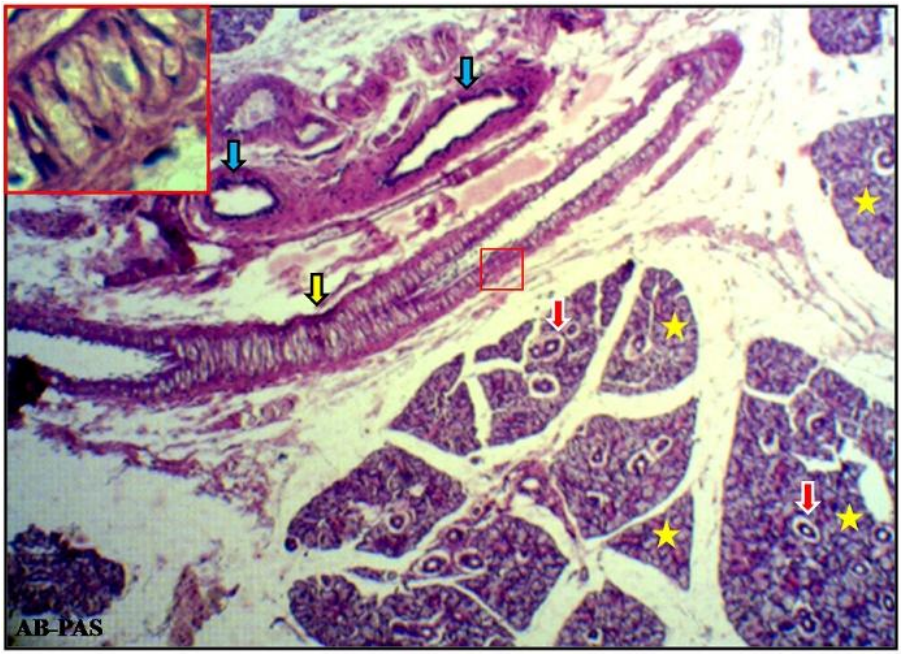

Fig. 14. Submandibular gland of local rabbit showed different parts of its duct system. It showed intralobular ducts (red arrows) inside the lobules (yellow stars), small and large interlobular ducts (blue arrows) and main excretory duct (yellow arrow). The figure showed many acini with blue and magenta colorations indicating positive reactions to AB and PAS, respectively, AB-PAS, X400 (red rectangle), X40 (the main figure)

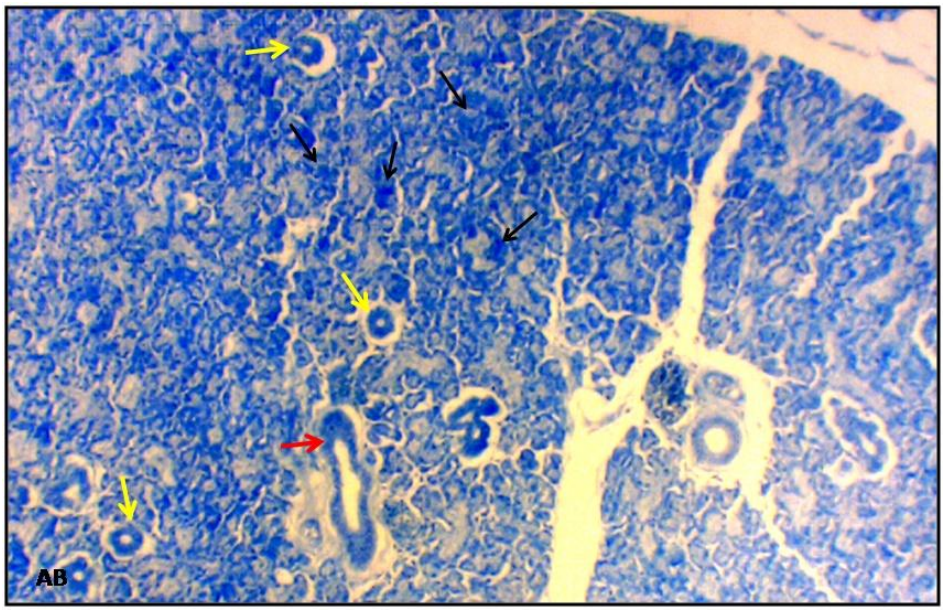

Fig. 15. Alcian blue (AB) stained section of submandibular gland of local rabbit. It showed positive reaction (dark blue) of the mucous acini (black arrows) indicating that the acini mucin is acidic and non sulfated type. The figure also showed intercalated (yellow arrows) and striated ducts (red arrow), AB, X40

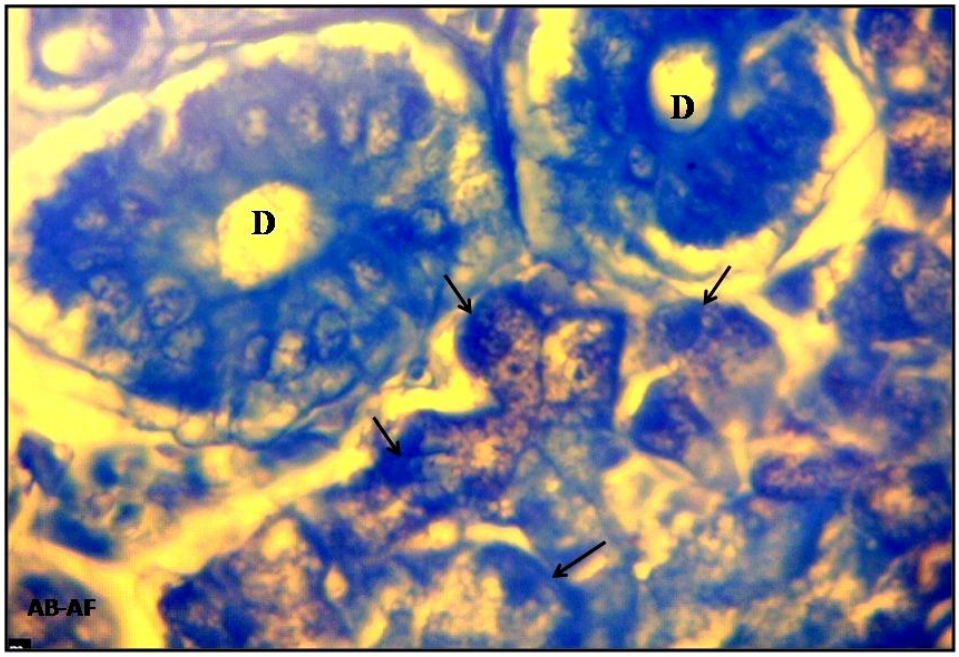

Fig. 16. Alcian blue – Aldehyde fuchsin (AB-AF) stained section of submandibular gland of local rabbit. It showed positive reaction (deep blue) to AB stain (black arrows) but negative reaction to the AF because mucous acini of the gland is of acidic and non sulfated type, AB-AF, X400

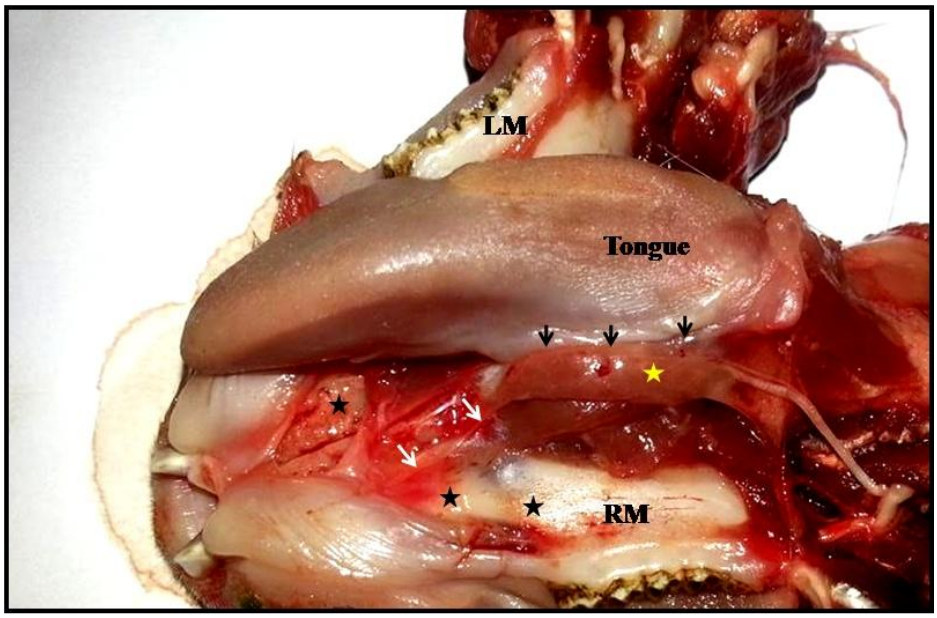

Fig. 17. Gross appearance of the sublingual gland of the local rabbit. The gland (yellow star) is located adjacent to the root of the tongue (black arrows). The figure showed the right mandible (RM), the main duct of the gland (white arrows) in the floor of the mouth cavity (black stars)

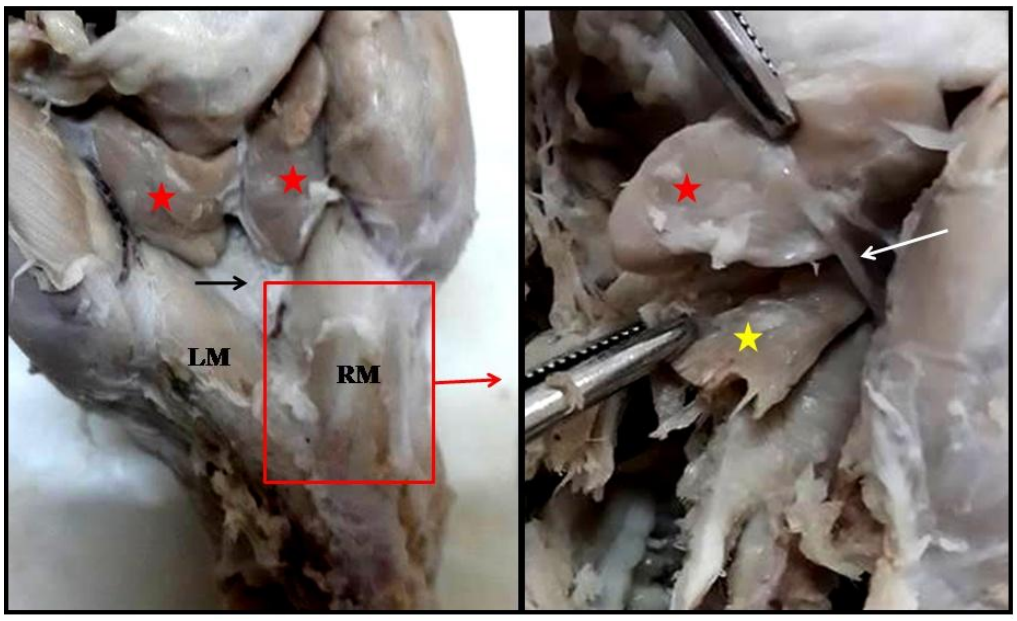

Fig. 18. Gross appearance of submandibular and sublingual glands of the local rabbit. Left panel: It showed the submandibular glands (red stars) located at the caudal part of the tongue, bounded cranially-laterally by the right and left mandibles (RM, LM) (black arrow). Right panel: It showed the sublingual gland (yellow star) situated rostrally to the submandibular gland which is visualized after the removing of the red rectangle area from the right mandible. The figure also showed the main duct of the submandibular gland (white arrow).

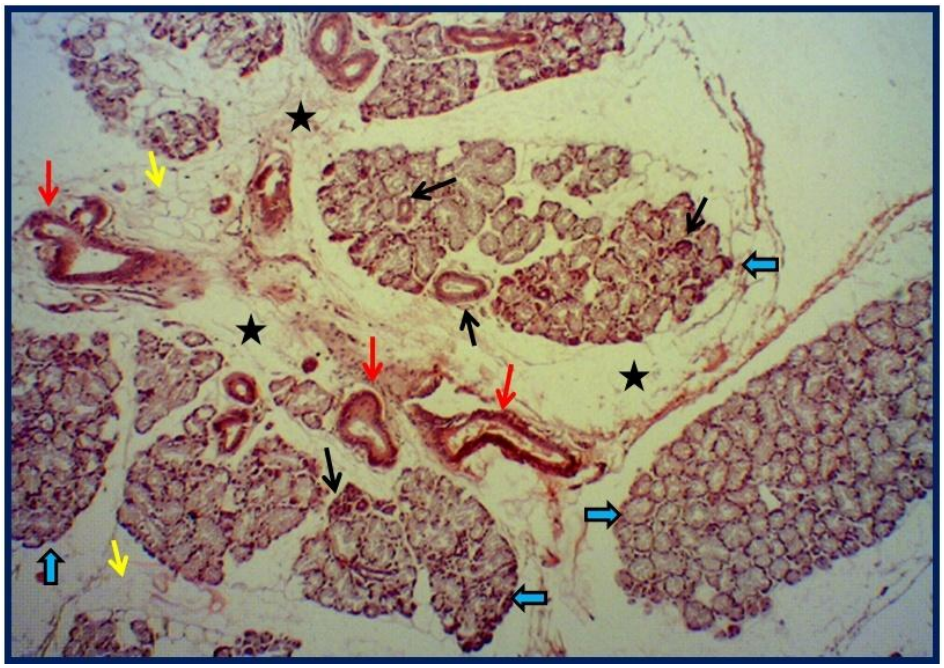

Fig. 19. Cross section in sublingual gland of the local rabbit. Thin connective tissue capsule (green arrows) which send interlobular septae (black stars) composed adipose tissue (yellow arrows) dividing the gland into many lobules (blue arrows). Many interlobular (red arrows) and intralobular ducts (black arrows) are present, H&E, X40

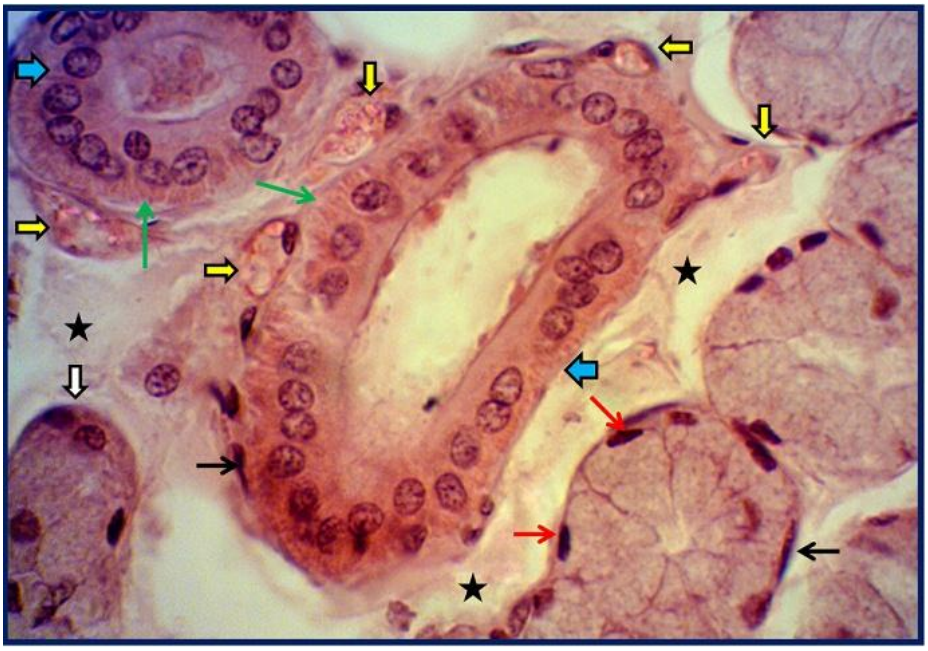

Fig. 20. Striated ducts and types of acini structured the sublingual gland of the local rabbit. The figure showed mucous acini characterized by pyramidal cell with flattened basally located nuclei (red arrows), serous demilune capped acinus (white arrow) and striated duct (blue arrow). The ducts are lined by simple columnar epithelium and showed basal striations (green arrows). Myoepithelial cells are found around the ducts and mucous acini (black arrows). Loose connective tissue intralobular septae (black stars) and many blood capillaries are present (yellow arrows), H&E, X400

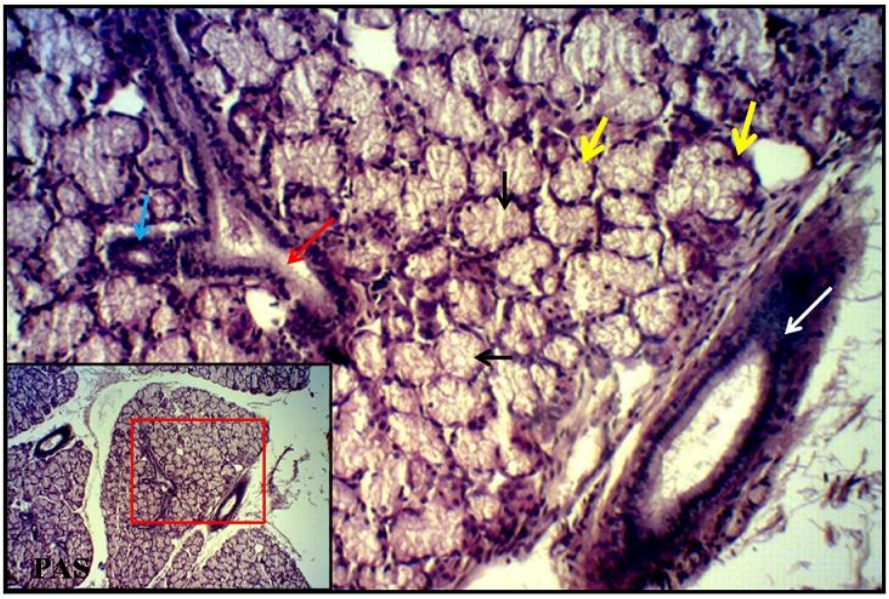

Fig. 21. PAS stained section of sublingual gland of the local rabbit. It showed positive reaction (magenta color) of the mucous acini (yellow arrows) which indicated the presence of acidic mucin. Positive reaction also observed in the interlobular duct (white arrow), and both intercalated (blue arrow) and striated (red arrow) intralobular ducts PAS, X40 (red rectangle), X100 (the main figure).

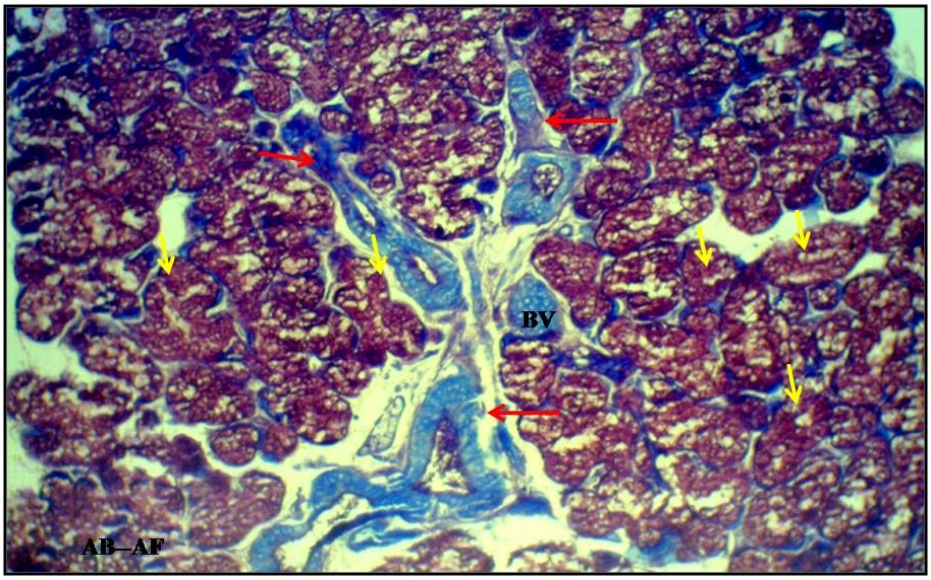

Fig. 22. Combined AB-AF stained section of the sublingual gland of local rabbit. showed positive reaction (dark purple) toward the AF indicated that the mucous acini are sulfated acidic mucin in nature (yellow arrows). Whereas, AB is negatively reacted with the mucin, because the stain is well reacted with non sulfated mucin only. The figure showed blood vessel (BV) and intralobular ducts (red arrows), AB-AF, X100

References

Abidu-Figueiredo, M., Xavier-Silva, B., Cardinot, T.M., Babinski, M.A. and Chagas, M.A. (2009). Celiac artery in New Zealand rabbit: Anatomical study of its origin and arrangement for experimental research and surgical practice. Pesq. Vet. Bras., 28(5): 237-240

Adnyane, I.K.M., Zuki, A.B., Noordin, M.M. and Agungpriyono, S. (2010). Histological study of the parotid and mandibular glands of barking deer (Muntiacus muntjak) with special reference to the distribution of carbohydrate content. Anat. Histol. Embryol., 39: 516–520

Amano, O., Mizobe, K., Bando, Y. and Sakiyama, K. (2012). Anatomy and histology of rodent and human major salivary glands. Acta Histochem. Cytochem., 45 (5): 241- 250

Al-Okaili, A. G., Sedeeq, B. I., Hazeem, M. I. (2008). Histological changes of the submandibular Salivary gland of mice maintained on a liquid diet. College of Dentistry, Tikrit University.,1- 4

Al- Sadi, S. (2013). Gross and radiological studies of the salivary gland in cattle. Bas. J. Vet. Res., 12(1): 65-75

Asari, M., Kimura, H., Ichihara, N., Kasuya, T. and Nishita, T. (2000). Immunohistochemistry of carbonic anhydrase isoenzymes (CA-I, II and III) in canine salivary glands. A distributional and comparative assessment. J. Vet. Med., 29: 9-12

Bazan, E., Watanabe, I., Lyomasa, M. M., Mizusaki, C. I., Sala, M. and Lobes, R. A. (2001). Morphology of the submandibular gland of gerbil. Rev. Chil. Anat., 19: 1

Bercier, M., Guzman, D. S-M., Stockman, J., Zwingenberger, A., Vapniarsky, N., Lowenstine, L., and Howkins, M. G. (2013). Salivary gland adinocarcinoma in domastic rabbit (Oryctolagus cunicolus). J. Exotic Pet Med., 22: 218- 224

Bhaskar, S. N., Lilly, G. E. and Bhussry, B. (1966). Regeneration of the salivary glands in the rabbit. J. Dent. Res., 45(1): 37- 41

Boshell, J. L. and Wilborn, W.H. (1983). Differences in the ultrastructure of the submandibular glands of baboon and Rhesus monkey revealed by the use of different fixatives. Cell Tissue Res., 231: 655-661

Brocco, S. L. and Tamarin, A. (1979). The topography of the rat submandibular gland parenchyma as observed with SEM. Anat. Rec., 194(3): 445- 459

Carberry, C. A., Flanders, J. A., Harvey, H. J. and Ryan, A. M. (1987). Salivary gland tumors in dogs and cats: a literature and case review. J. Am. Anim. Hosp. Assoc., 24: 561–567

Chauncey, H. H., Feller, R. P. and Henriques, B. L., (1966). Comparative electrolyte composition of parotid, submandibular and sublingual secretions. J. Dent. Res., (45)4: 1230

Coire, F. A. S., Umemura, A. L. O., Cestari, T. M. and Taga, R. (2003). Increase in the cell volume of the rat submandibular gland during postnatal development. Braz. J. morphol. Sci. 20(1): 37-42

Cope, G. H., Pratten, M. K. and Williams, M. A. (1976). Correlative morphological and

biochemical study of the effects of isoprenaline on the organelle and membrane content of the

rabbit parotid gland. Histochem. J., 8: 404-418

Cotroneo, E., Proctor, G. B. and Carpenter, G.H. (2010). regeneration of acinar cells following ligation of rat submandibular gland retraces the embryonic-perinatal pathway of cytodifferentiation. Differentiation., 79: 120- 130

Dehghani, S. N., Tadjalli, M. and Masoumzadeh, M. H. (2000). Sialography of sheep parotid and submandibular salivary glands. Res. Vet. Scie., 68: 3-7

Dehghani, S. N., Tadjalli, M. and Seifali, A. (2005). Sialography in horse: technique and normal appearance. VETERINARSKI ARHIV 75 (6), 531-540

Eifler, A. C., Lewandowski, R. J., Virmani, S., Chung, J. C., Wang, D., Tang, R., Szolc-Kowalska, B., Woloschak, G. E., Mulcahy, M. F., Ryu, R. K., Salem, R., Larson, A. C., Cheon, E., Strouch, M., Zarlengo, D., Bentrem, D. and Omary, R. A. (2009). Development of the VX2 Pancreatic Cancer Model in Rabbits: A Platform to Test Future Interventional Radiology Therapies. Society of Interventional Radiology 34th Annual Sci. Meeting

Elewa, Y.H., Bareedy, M.H., Abuel-Atta, A.A., Ichii, O., Otsuka, S., Kanazawa, T., Lee, S., Hashimoto, Y. and Yasuhiro Kon, Y. (2010). Structural characteristics of goat (Capra hircus) parotid salivary glands. Jpn. J. Vet. Res., 58(2): 121-135

Elewa, Y. H. A., Ichii, O., Otsuka, S., Hashimoto, Y. and Kon, Y. (2014). Structural change of goat parotid salivary gland: pre- and -post weaning periods. Anat. Histol. Embryol., 43(4): 265-272

Ekele, I., Uchenna, N., Okechukwu, N. and Isaiah, A. (2013). Histology of the parotid salivary gland of the African Palm Squirrel. Rev. Fac. Cs. Vets. University of Agriculture Umudike, Abia State, Nigeria., 54 (1): 11- 16

El-Kordy, E. A., Alanazi, A. D., Ali, S. S., Makhlouf, M. M. M. and Rabah, S. O. (2014). Histological, Histochemical and uItrastructural changes in the submandibular gland of starved young male cats. J. Cytol. Histol., 5 (4): 1-9

Estecondo, S., Codon, S. M. and Casanave, E. B. 2005. Histological study of the salivary glands in Zaedyus pichiy (Mammalia, Xenarthra, Dasypodidae). Int. J. Morphol., 23: 19-24

Fernandes, A. C. S., Lima, R. G., Rossi, M. A. and Márcio Cajazeira Aguiar, M. C. (2009). Parotid gland with double duct: an anatomic variation description. Int. J. Morphol., 27(1): 129-132

Ghoshal, N. G. and Bal, H. S. (1989). Comparative morphology of the stomach of some laboratory mammals. Lab. Anim. J., 23: 21 – 23

Habata, I., Yasui, T., Fujimori, O. and A. Tsukise, A. (2012). Immunohistochemical aspects of anti-microbial properties in goat submandibular glands. Anat. Histol. Embryol., 41: 54–59

Haghighat, N. and Al-Hashimi, I. (1999). A pilot study on the effect of radiation on calmodulin in rat submandibular salivary glands. Arch. Oral Biol., 44: 383-389

Hakim, S. G., Lauer, I., Kosmehl, H., and Sieg, P. (2002). The superficial mandibular gland of the rabbit: a new experimental model for scintigraphic evaluation of salivary glands. Int. J. Oral Maxillofac. Surg., 31: 303- 308

Harrison, J.D., Auger, D.W., Paterson, K.L. and Rowley, P.S.A. (1987). Mucin histochemistry of submandibular and parotid salivary glands of man: Light and electron microscopy. Histochem. J., 19: 555-564

Head, K. W. (1976). (I) Tumors of the upper alimentary tract, (II) Tumors of the lower alimentary tract. Bull. World Health Organ., 53: 2 - 3

Holsinger, F.C. and Bui, D.T. (2007). Anatomy, function, and evaluation of the salivary glands. In: Myers EN, Ferris RL editor. **Salivary gland disorders.** Berlin Heidelberg: Springer-Verlag; 1–16

Ikpegbu, E., Nlebedum, U. C., Nnadozie, O. and Agbakwuru, I. O. (2013). The submandibular salivary gland microscopic morphology of the adult African giant pouched rat (Cricetomys gambianus, waterhouse-1840). I. J. V. M., 7(2): 117- 122

Jacob, S. and Poddar, S. (1977). Gross and microscopic anatomy of the major salivary glands of the ferret. Acta. Anat., 98: 434- 443

Jaskoll, T., Zhou, Y.M., Chai, Y., Makarenkova, H.P., Collinson, J.M., West, J.D., Carvalho, A.D. (2002). Embryonic submandibular gland morphogenes is; Stage- specific protein localization of FGFs, BMPs, Pax6 and Pax9 in normal mice and abnormal phenotypes in FgfR2- III c(t/Delta), BMP7(-/-) and Pax6(-/-) mice. Cells. Tissues. Organs, 170:83-90

Jonjic, S. (2001). Surgical removal of mouse salivary glands. Curr. Protoc. Immunol. Chapter 1; Unit 1.11

Khojasteh, S.M.B. and Delashoub, M. (2012). Microscopic anatomy of the parotid and submandibular salivary glands in European hamster (Cricetus cricetus L.). Internat. Res. J. Appl. Basic Sci., 3(7): 1544-1548

Kim, S. K. (1976). The cytochemical localization of adenylate cyclase activity in mucous and serous cells of the salivary gland. J. Supramolecular Structure 4: 185 (145)-197 (157)

Kimura, J., Habata, I., Endo, H., Rerkamnuagchoke, W., Kurohmaru, M., Yamada, J., Nishida, T., Tsukise, A. (1998). Histochemistry of comoplex carbohydrate in the major salivary glands of Hoary Bamboo rats (Rhizomys purinosus). Anat. Histol. Embryol., 27: 147-153

Lebenthal, E. (1987). Role of Salivary Amylase in Gastric and Intestinal Digestion of Starch. Dig. Dis. Sci., 32(10): 1155-1157

Liu, P., Denny, P.A. and Denny, P. (2000). The effect of ageing on parenchymal cell populations in adult female mouse submandibular gland. Archiv. Oral Biol., 45: 585-592

Luna, G. (1968): ″Manual of Histological Staining Methods of the Armed Forced Institute of pathology″. 3rd Ed. McGraw Hill book Co. New York, Pp: 71, 74, 98

Maženský, D., Vdoviaková, K., Petrovová, E. and Luptáková, L. (2009). Variability in origin of some branches of aorta thoracica in Rabbit. Slovak J. Anim. Sci., 42, supplement 1: 60-62

McLaughlin, C. A. and Chiasson, R. B. (1990). Laboratory anatomy of the rabbit, 3rd ed. Brown Publishers, Washington DC; 59-80

Mizuno, T., McKinnon, A., Ichihara, N., Amasaki, T., Asart, M., Nishita, T., Oishi,, M., Soeta S. and Amasaki, H. (2009). Histological structure and distribution of carbonic anhydrase isozymes in major salivary gland. Anat. Histol. Embryol., 38: 449- 454

Munyala, R., Liumsiricharoen, M., Pongket, P., Prapong, T. and Apinun suprasert, A. (2008). Characterization of glycoconjugates in the sublingual salivary gand of Malayan Pangolin (Manis javanica). Kasetsart J. (Nat. Sci.), 42 : 88 - 94

Miyazaki, T., Tatsukawa, S., Kitamura, H., Ina, K., Abe, H. and Fujikura, Y. (2008). Morphological and functional changes of the rat parotid glandular cells by clipping and reopening the parotid duct, using HAM8 antibody. Anat. Sci. Internat., 83: 89–95

Ogawa , Y. (2003). Immunohistochemstry of epithelial cells in salivary gland. Histochemical. cytochemical., 38: 343-426

Pedini, V., Ceccarelli, P. and Gargiulo, A.M. (1994). Glycoconjugates in the mandibular salivary gland of adult dogs revealed by lectin histochemistry. Res. Vet .Sci., 57: 353-357

Pratten, M. K., Williams, M. A. and Cope, G. H. (1977). Compartmention of enzyme in the rabbit of parotid salivary gland a study by enzyme histochemical tissue fractionation and morphometric techniques. Histochem. J ., 9: 573-598

Rana, R., Minhas, L. A., Saga, Z. and Mubarik, A. (2012). Histological study of human sublingual with special emphasis on intercalated and striated ducts. Army Medical College, 4:1- 8

Raubenheimer, E.J., van Niekerk, J.P. and Hauman, C.H.J. (1987). Salivary myoepithelium: distribution, structure, functions and pathologic proliferations. J. Dent. Assoc. South Africa, 42: 631-637

Redman, R. S. (1994). Myoepithelium of salivary glands. Microsc. Res. Techn., 27: 25- 45

Rosa, F., Basso, L., Valdora, F., Neumaier, C. E.and Baio, G. et al, (2014). Normal mouse anatomy on 3 T MRI. EPOS. Doi: 10.1594/ecr2014/C-1198

Sato, A., Goto, F. and Miyoshi, S. (1994). Ultrastructure of the main excretory duct epithelium of the female mouse submandibular gland with special reference to sexual dimorphism. Cell Tissue Res., 277: 407-415

Shackelford, J.M. and Schneyer, C.A. (1964). Structural and functional aspect of rodents salivary gland including two desert species. Am. J. Anat., 155: 279-307

Sozmen, M., Brown, P. J. and Cripps, P. J. (1999). Quantization of histochemical staining of salivary gland mucin using image analysis in cats and dogs. Vet. Res., 30: 99- 108

Stephens, L. C., King, G. K., Peters, L. J., Ang, K. K., Schultheiss, T. E. and Jardine, J. H. (1986). Unique radiosensitivity of serous cells in Rhesus monkey submandibular glands. A. J. P., 124 (3): 479-487

Suzuki, S., Kamei, K. and Otsuka, J. (1975). On the fine structure of salivary glands of goat and dog: I. Parotid gland. Bull. Fac. Agr. Kagoshima Univ., 25: 25-41

Suzuki, S. and Otsuka, J. 1978. On the fine structure of salivary gland of horse: I. parotid gland. Bull. Fac. Agr. Kagoshima Univ., 28: 15-24

Suzuki, S., Ago, A., Mohri, S. et al (1983).Fine structure of the parotid gland of Djungarian

hamster (Phodopus sungarus). Jikken Dobutsu, 32(4): 175-184

Tandler, B., Nagato, T.and Phillips, C.J.(1998). Ultrastructure of the binary parotid glands in the free-tailed bat Tadarida thersites. II. Accessory parotid gland. Anat. Rec., 251:122–135

Tadjalli, M., Dehghani, S. N. and Ghadiri , M. (2002). Sialography of goat parotid, mandibular and sublingual salivary glands. Small Ruminant Research, 44: 179-185

Triantafyllou, A., Fletcher, D. and Scott, J. (1999). Morphological phenotypes and functional capabilities of submandibular parenchymal cells of the ferret investigated by protein, mucosubstance and enzyme histochemistry. Histochem. J., 31: 789-796

Triantafyllou, A., Fletcher, D. and Scott, J. (2005). Organic secretory products, adaptive responses and innervation in the parotid gland of ferret: A histochemical study. Archiv. Oral Biol., 50: 769-777

Tsuboi, T., Honda, T., Hishida, S., Shijetomi, T., Ueda, M. and Sugiura, Y. (2004). A quantitative study of nerve fibers density in the submandibular gland of rats. Nagoya. J. Med. Sci., 67: 25- 34

Tucker, A. S. (2007). Salivary gland development. Semin. Cell. Dev. Biol., 18(2): 237- 244

USDA (United States Department of Agriculture), 2006. Fiscal Year 2006 AWA Inspections (accessed October 2009, at: http://www.aphis.usda.gov. /animal welfare/ /awreports/awreport2006.pdf/).

Van- Lennep, E. W. and Young, J. A. (1978). The Morphology of Salivary Glands. Academic Press; London

Wang, S. L., Li, J., Sun, K., Liu, X. Y., and Zhang, Y. G. (1998). Sialographic characterization of the normal parotid gland of the miniature pig. Dentomaxillofacial Radiol., 27: 178- 181

Watanabe, I., Seguchi, H., Okada, T., Kobayashi, T., Jin, Q. S. and Jiang, X.D. (1996). Fine structure of the acinar and duct cell components in the parotid and submandibular salivary glands of the rat: a TEM, SEM, and HRSEM study. Histol. Histopathol., 11: 103- 110

Weidner, S., Probst, A., and Kneissl, S. (2012). MR Anatomy of Salivary Glands in the Dog. Anat. Histol. Embryol., 41: 149- 153

Xin, Z., Jun, L., Xiao-yong, L., Yi-lin, S., Chun-mei, Z., Song-ling, W. (2005). Morphological characteristics of submandibular glands of miniature pig. Chin. Med. J., 118 (16): 1368- 1373

Yazdani Moghaddam, F., Darvish, J., Mahdavi Shahri, N., Abdulamir, A.S., Mousavi, M. and Daud, S.K. (2009). Comparative histological and histochemical inter- species investigation of mammalian submandibular salivary glands. Res. J. Appl. Sci., 4(1): 50-56

Zainuddin, N., Adnyane, I. K. M. Sari, D. K. Wresdiyati, W.and Agungpriyono, S. (2000). Histological and histochemical study of the submandibular and parotid glands of the tree shrew (Tupaia glis) with special reference to the distribution of carbohydrate. Primatol. Indonesia 3, 9–16.

Zhou, J., Wang, H., Yang, G., Wang, X., Sun, Y., Song, T., Zhang, C. and Wang, S. (2010). Histological and Ultrastructural characterization of developing miniature pig salivary glands. The Anat. Record., 293: 1227- 1239