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Research Article

MACRO AND MICROSCOPIC CHARACTERISATION OF MAJOR SALIVARY GLANDS IN WISTAR RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

The Wistar rat possessed three pairs of well-developed major salivary glands; mandibular, parotid and sublingual. The histo-architecture and function of major salivary glands differ with species, diet and their type. The mandibular salivary glands were the largest and oval in shape. They were observed in the ventral cervical region adjacent to the respective external jugular veins. The parotid salivary glands were observed at the base of the auricular concha and extended distally up to the aboral border of them and mandibular glands on either side. The sublingual salivary glands were observed antero-laterally at the rostral pole of the mandibular glands. The mandibular glands were of the mixed type with % predominantly serous and with few mucous type acini. Histochemically, the mucous type acini were negative for mucin whereas the serous acini showed a mild positive reaction to PAS in the basement membrane. The parotid contained serous acini and the granules seen in the cytoplasm of acinar cells showed negative reaction to PAS and Alcian blue.The sublingual gland consisted of predominantly mucous acini and showed moderate positive reaction to PAS and positive reaction to Alcian blue.The serous demilunes were more at the periphery of the gland and were located at the bases of the mucous acini.

Keywords: Gross morphology, Histology, Major salivary glands, Rat

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Mammals possess well developed major salivary glands (submandibular, parotid, sublingual and zygomatic glands). The glands develop from ectodermal lining of the primitive oral cavity (Poddar and Jacob, 1977). Salivary glands provide lubrication for eating and vocalisation. Salivary secretions of enzymes initiate the digestion of carbohydrates. It also controls bacterial flora through the secretion of lysozyme (Genkins, 1978). Some experiments have demonstrated that it is involved in immunological response through IgA immunoglobulin. It also secretes potassium and reabsorbs sodium (Ferraris *et al.*, 1999). Specifically, the salivary glands of rodent are important with regard to adaptations to diets, environments and taxonomic studies (Stimson *et al.*, 2007).

The literature on morphology and histology of the major salivary glands in rat was limited. Therefore, the present work was planned to provide baseline data that may be useful in knowing the pathophysiology of diseases of salivary glands, and to aid in nutritional studies.

MATERIALS AND METHODS

Twenty clinically healthy male Wistar rats were used in this study (approved by IAEC vide No. 22 /IAEC/2019 Dated: 14/12/19). The rats were maintained and sacrificed as per ethical guidelines.The work was carried out in the Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram. After gross observations of salivary glands, the tissue samples were fixed in 10% Neutral buffered formalin and Bouin's fixatives and the samples will be subjected to standard processing technique for routine histological studies (Luna, 1968).

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RESULTS AND DISCUSSION

Morphology: There are three paired major salivary glands in rats *viz.*, mandibular sublingual and parotid (Fig. 1). These findings were similar to the observations of Amano (2011)in mouse. The mandibular salivary glands were the largest and were oval in shape. They were located in the ventral cervical region adjacent to the respective external jugular veins. The sublingual salivary glands were of lighter colour and were located antero-laterally at the rostral pole of the mandibular glands (Fig. 1). The two mandibular ducts (Wharton's ducts) left the anterior ends of the two glands and emptied into the ventral buccal cavity. The duct of sublingual salivary glande merged from its cranial end and ran parallel to the Wharton's duct (Fig. 2). These findings are in accordance with the observations of Hunt (1925) in rats and Hummel *et al.* (1966) in mice. The mandibular lymph node was in direct contact with the mandibular gland. These findings are in accordance with the reports of Matosz *et al.* (2010).

The parotid salivary glands were loosely organised and located at the base of the auricular concha and extended distally up to the aboral border of them and mandibular glands on either side. The parotid gland had two distinct parts. The first portion was located above the base of the outer ear, disk-shaped, with the medial side being slightly concave, yellow-brown coloured, and the second one surrounded the first in a ventral way (Figs. 1 & 2). These findings were similar with findings of Matosz *et al.* (2010).

The duct formed from the different parts of the parotid gland passed on the surface of the masseter muscle, then turned inward in to the mouth cavity. These findings were similar to Matosz *et al.* (2010) in rats. Cranial to the parotid gland, a disk-shaped gland was observed. The length of this gland was only up to the proximal half compared to the length of the parotid gland and called extraorbital lacrimal gland (Fig. 1). The gland can be easily noticed with a darker colour than the parotid gland (Fig. 1). These observations are in agreement with the findings of Da Cunha *et al.* (2004).

Microscopic features: All the three pairs of major salivary glands were compound tubulo-alveolar glands. They were separated into lobules by connective tissue septa and the alveoli were lined by secretory pyramidal cells. The mandibular gland was a mixed gland in which the serous units were predominant than the mucous acini whereas (Fig. 3), the sublingual gland also showed mixed acini with predominantly mucous ones (Fig. 9). These observations are in accordance with the reports in giant pouched-rat (Ikpegbu *et al.*, 2013a) and armadillo (Zaedyspichiy) (Esteconodo *et al.*, 2005). Purely mucous acini have been reported in the submandibular gland of ferrets (Poddar and Jacob, 1977).

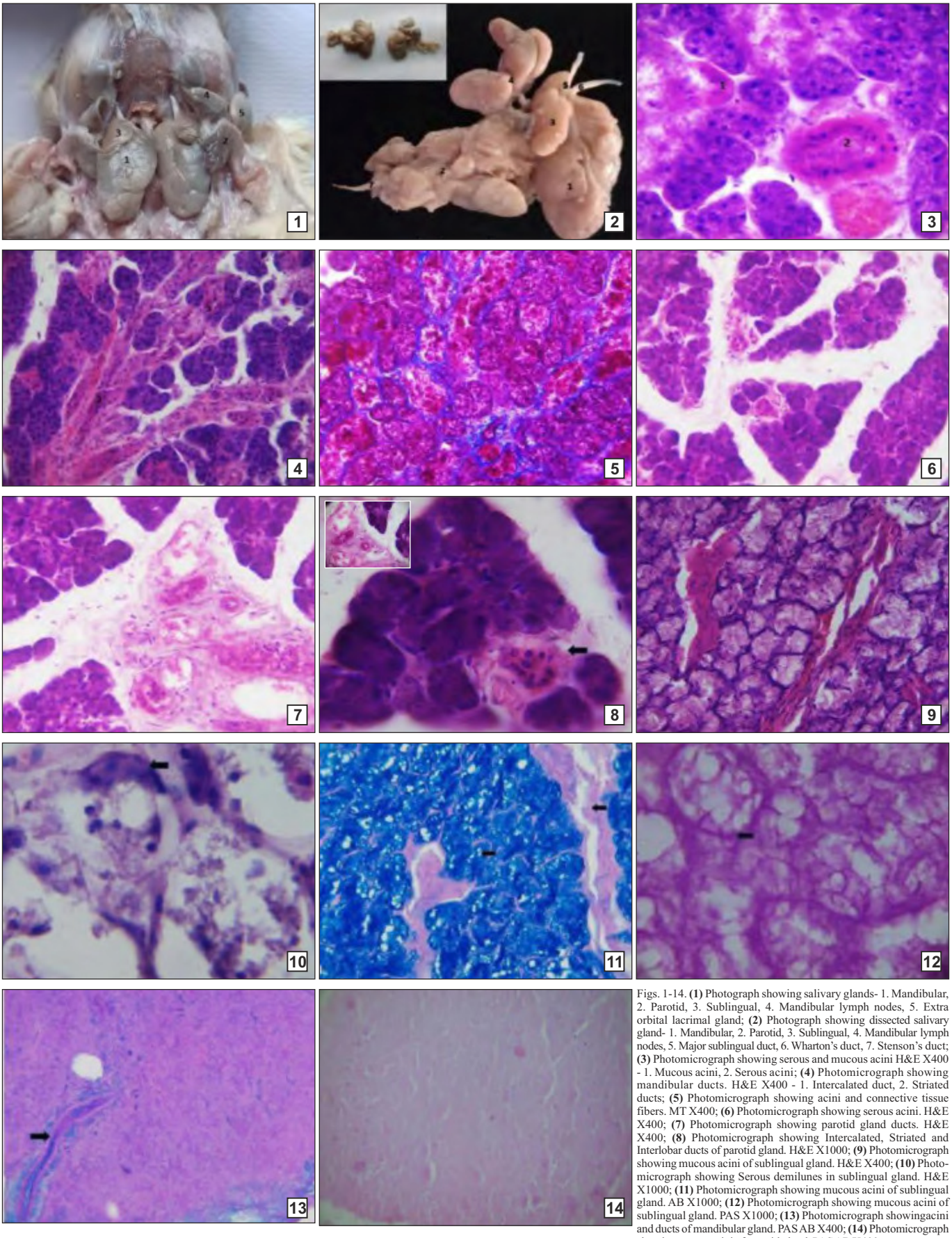
Mandibular salivary gland: The histological observations revealed that the mandibular gland was covered by a thin connective tissue capsule. The parenchyma was separated into lobules by connective tissue septa and the alveoli were lined by secretory cells.The mandibular and sublingual glands were enclosed in a common connective tissue capsule with a very thin connective tissue strand that separated them. It was a mixed gland and the serous units were predominant than the mucous acini. The serous cells were lightly basophilic, granular, with rounded or spheroidal nuclei placed more centrally. In the interlobular connective tissue of the gland, blood vessels and nerves were present (Figs. 3 & 4). Similar findings were reported in giant pouched rats by Igbokwe *et al.* (2015)

The alveoli were continuous with the terminal intercalated ducts (Fig. 4) flattened or cuboidal cells, which formed larger intralobular ducts with cuboidal or columnar cells containing basal striations. The interlobular ducts were very well developed. The interlobular ducts and main excretory ducts were lined with columnar or stratified columnar epithelium except at the orifices, where they were lined by stratified squamous epithelium (Fig. 4). Connective tissue fibres were observed between the mucous and serous acini (Fig. 5). Myoepithelial cells with flattened nuclei were sometimes identified on the outer layer of the acinar secretory cells and in the proximal portion of the ductal system, especially intercalated duct (Igbokwe *et al.*, 2015). In contrast to this, in the present study there were no serous demilunes observed in mandibular glands.

Histochemically, the serous acini showed a mild positive reaction to Periodic acid- Schiff (PAS) in the basement membrane.The intralobular duct (striated ducts) contents were PAS negative and reacted moderatelyto Alcian blue (AB). The serous and mucous acini were AB negative. No demilunes were observed in this gland as confirmed by histochemical staining properties (Fig. 13). These findings were slightly similar except that few scattered mucous acini were strongly AB positive, as reported in giant pouched rats by Igbokwe *et al.* (2015). The presence of well-developed serous secretory units might have needed for increased breakdown of solid carbohydrates diet by amylase and lysozyme production (an antibacterial agent) may help to ward off infectious agents.

Sublingual salivary gland: The small sublingual glands were divided into lobules by the connective tissue septae projected from the capsule. These lobules composed of acini containing tall pyramidal cells with basally located nuclei and pale to blue-staining cytoplasm with H&E. Distinct striated ducts were rarely observed. Serous demilunes (Crescent of Gianuzzi) were clearly observed (Figs. 9 & 10). It was predominantly mucous in the current study as in most rodents, ruminants and swine (Pinkaff, 1993). In both species, well developed striated ducts (intralobular) with only few intercalated ducts were encountered, similar to that of desert rats and antelope squirrel (Shackleford and Schneyer, 1964). Histochemically, the mucous acini and the luminal contents of all grades of ducts in the gland reacted strongly to AB staining (Fig. 11). The serous demilunes were intensely stained with PAS (Fig. 12). Similar findings were reported in giant pouched rats by Igbokwe *et al.* (2015).

Parotid salivary gland: The parotid salivary glands of the rat were serous glands, as also elucidated in mice by Gude (1982). The alveoli were small and composed of three or four secretory pyramidal cells. The nuclei were basally located and the cytoplasm was basophilic with H&E staining (Fig. 6). It was a well-developed lobulated gland with typical serous acini and several intermingled intralobular (intercalated and striated ducts) and larger interlobular ducts (Fig. 7 & 8). These features were similar to that reported in many rodents and mammals including European hamster (Khojasteh and Delashoub, 2012), giant rats (Asojo and Aire, 1983), African palm squirrel (Ikpegbu *et al.*, 2013b), rabbits (El-Ramli *et al.*, 2013) and



Figs. 1-14. **(1)** Photograph showing salivary glands- 1. Mandibular, 2. Parotid, 3. Sublingual, 4. Mandibular lymph nodes, 5. Extra orbital lacrimal gland; **(2)** Photograph showing dissected salivary gland- 1. Mandibular, 2. Parotid, 3. Sublingual, 4. Mandibular lymph nodes, 5. Major sublingual duct, 6. Wharton's duct, 7. Stenson's duct; **(3)** Photomicrograph showing serous and mucous acini H&E X400 - 1. Mucous acini, 2. Serous acini; **(4)** Photomicrograph showing mandibular ducts. H&E X400 - 1. Intercalated duct, 2. Striated ducts; **(5)** Photomicrograph showing acini and connective tissue fibers. MT X400; **(6)** Photomicrograph showing serous acini. H&E X400; **(7)** Photomicrograph showing parotid gland ducts. H&E X400; **(8)** Photomicrograph showing Intercalated, Striated and Interlobar ducts of parotid gland. H&E X1000; **(9)** Photomicrograph showing mucous acini of sublingual gland. H&E X400; **(10)** Photomicrograph showing Serous demilunes in sublingual gland. H&E X1000; **(11)** Photomicrograph showing mucous acini of sublingual gland. AB X1000; **(12)** Photomicrograph showing mucous acini of sublingual gland. PAS X1000; **(13)** Photomicrograph showingacini and ducts of mandibular gland. PAS AB X400; **(14)** Photomicrograph showing serous acini of parotid gland. PAS AB X400.

armadillos (Zedyuspichiy) (Estecondo *et al.*, 2005). However, the parotid of some carnivorous mammals including dog, cat and ferret was reported to be sero-mucous (Poddar and Jacob, 1977). Histochemically, the serous acini and ducts in rats were negative for PAS-AB (Fig. 14).

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