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Thymus of Buffalo: Ultra Structural Perspective

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ABSTRACT

Ultrastructural features of the thymus were studied in five young buffalo with age ranges from 1 to 1.5 years old. In the cortex, densely packed thymocytes (lymphocytes) in various stages of differentiation were observed. The shapes of thymocytes were round to elliptic in outline. The cytoplasm was poor in terms of organelles. Thymic epithelial cells (TEC) were also observed throughout the cortex. They were characterised by pale, electron-lucent cytoplasm, irregular shape with long cytoplasmic processes connected to each other by desmosomes to form a reticular network. In medulla, a loose arrangement of mature thymocytes, and medullary thymic epithelial cells (TECs) were observed. There was degenerative nuclear and cytoplasmic structure in the central part of Hassall's corpuscles. Additionally, other cell types within the thymus, including macrophages and mast cells, which contribute to the phagocytic clearance of apoptotic thymocytes and antigen presentation were also observed.

Key words: Buffalo, Thymus, Thymic epithelial cells, Transmission electron microscope.

The thymus represented the central organ of the immune system, as it provides the micro-environment required for the development of T lymphocytes. Thymic epithelial cells (TECs) are among the most critical components in the thymic microenvironment supporting thymocyte selection and maturation. The thymus had been studied earlier in buffalo (Rammaya *et al*., 2011), Egyptian water buffalo (Roshdy and Derbalah, 2017), pig (Ceylan and Alabay, 2017), dog (Bodey *et al*., 1987), one humped camel (Roshdy, 2009), Arabian camel (Aly, 1988), goat (Prasad *et al*., 2012 and Mainde *et al*., 2018), Surti goats (Chaurasia & Menaka, 2024) and Nandanam chicken (Kannan *et al*., 2015). Keeping in view the importance of organ, present study has been envisaged to explore the ultrastructural details of thymus of the buffalo.

MATERIALS AND METHODS

In the present study, tissue samples of 1 mm^3 were collected from the thymus of 05 young buffaloes of non-descript breed, age ranged between 1 to 1.5 years from slaughter house immediately after slaughter. The fresh collected samples were thoroughly washed in chilled 0.1M phosphate buffer (pH 7.4) saline, fixed in 2.5% glutaraldehyde. Following post fixation, the tissues were again washed in phosphate buffer saline, processed and resin blocks were prepared. Semi-thin sections of 0.5-2.0 µm were cut for scanning under optical microscope. After scanning, 70-90 nm ultra-thin sections were cut by diamond knife ultra-microtome and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film. The grids were stained with uranyl acetate followed by lead citrate and examined under transmission electron microscope (TEM) at AIIMS, New Delhi.

RESULTS AND DISCUSSION

The thymus was broadly divided into cortical and medullary regions, each characterized by distinct cellular populations and morphologies as observed through Transmission Electron Microscope (TEM). In the cortex, densely packed thymocytes (lymphocytes) in various stages of differentiation were observed (Fig. 1) as reported earlier in buffalo (Rammaya *et al*., 2011), goat (Sivagnanam *et al*., 2017), camel (Aly *et al*., 1988) and pig (Ceylan and Alabay, 2017). The shapes of thymocytes were round to elliptic in outline as observed earlier in goat (Sivagnanam *et al*., 2017). In nucleus heterochromatin was relatively more, darkly stained and distributed peripherally, whereas lightly stained active euchromatin was seen towards the centre of the nucleus (Fig. 2) as reported earlier in buffalo

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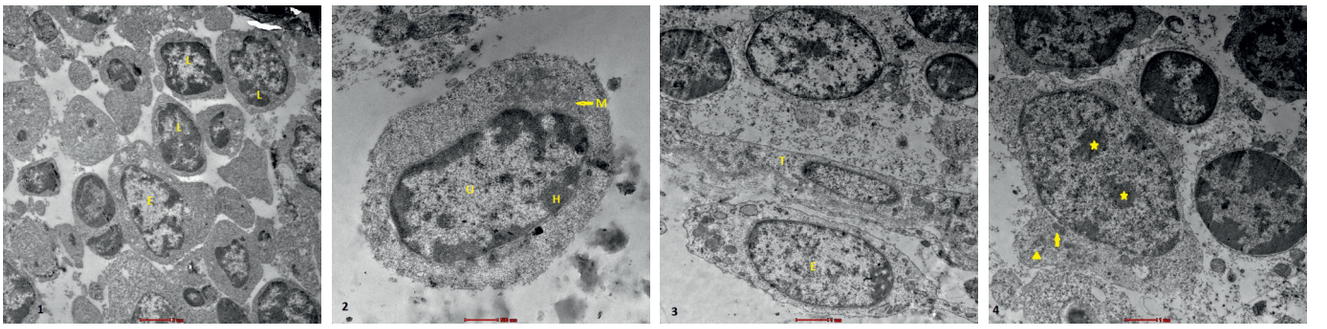

Fig. 1: Transmission electron micrograph of cortex of buffalo thymus depicting thymocytes (L) and epithelial reticular cell (E). 2µm; **Fig. 2:** Higher magnification depicting euchromatin (U) and heterochromatin (H) in the nucleus of thymocytes, mitochondria (M). 500nm; **Fig. 3:** Showing epithelial reticular cell (E) and trabecular cells (T). 1µm; **Fig. 4:** Showing polyribosomes (arrow), mitochondria (arrow head) and nucleolus (star) in the epithelial reticular cell. 1µm.

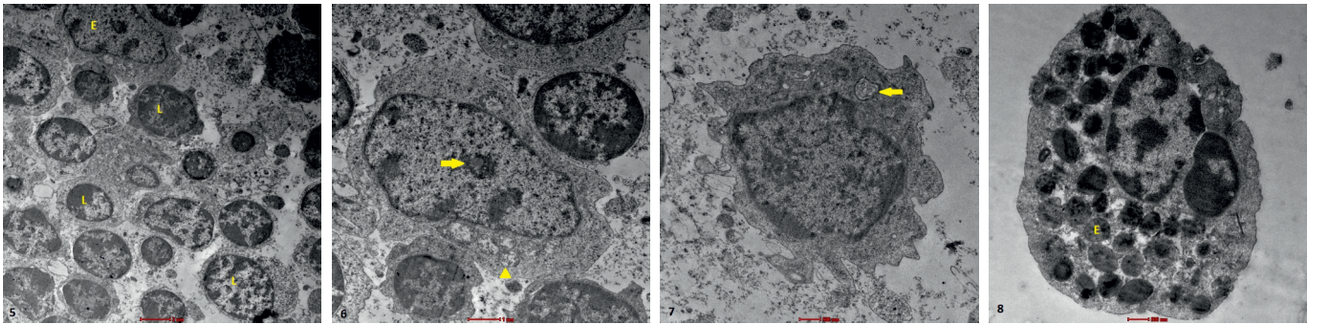

Fig. 5: Transmission electron micrograph of medulla of buffalo thymus showing epithelial reticular cell (E) and thymocytes (L). 2µm; **Fig. 6:** Showing nucleolus (arrow), mitochondria (arrow head) in the epithelial reticular cell. 1µm; **Fig. 7:** Showing cell debris/ structures phagocytise (arrow) in the macrophage. 500nm; **Fig. 8:** Showing large number of vacuole containing variable electron dense granules (E) in the mast cell 500nm.

(Rammaya *et al*., 2011), Egyptian Water buffalo (Roshdy and Derbalah, 2017) and one humped camel (Roshdy, 2009). The thymocytes showed electron-dense appearance close to the nuclear membrane with a little ring shaped cytoplasm. The cytoplasm was poor in terms of organelles. The cortex also had lymphocytes with smaller nuclei. Similar finding were observed in one humped camel (Roshdy, 2009). In electron micrograph the small lymphocytes showed more amount of cytoplasm. Thymic epithelial cells (TEC) found in the cortex were seen to have differences in terms of morphology and placement. A few TEC cells were located to form an external epithelium barrier in subcapsular, trabecular (Fig. 3) and perivascular areas as already reported in goat (Sivagnanam *et al*., 2017). Some TEC cells were scattered throughout the cortex. These cells have round to elliptic or elongated nuclei with finely dispersed low electron density chromatin and a prominent nucleolus (Fig. 4). They were characterised by pale, electron-lucent cytoplasm, having poly- ribosomes, numerous small mitochondria, rough endoplasmic reticulum and irregular shape with long cytoplasmic processes (Fig. 4) in accordance with observation in one humped camel (Roshdy, 2009) and Nandanam chicken (Kannan *et al.,* 2015)

In medulla, the thymocytes, and medullary thymic epithelial cells (TECs) were loosely arranged (Fig. 5) as reported earlier in pig (Ceylan and Alabay, 2017) and camel (Aly *et al*., 1988). The morphologies and characteristic of cellular details of the lymphocyte in medulla was similar as observed in cortex. It was observed that the TECs were cells with pale appearance in terms of electron density. The nucleus appeared irregular with a prominent nucleolus (Fig. 6). The cytoplasm displayed free ribosomes, numerous small mitochondria, rough endoplasmic reticulum and vacuoles of variable sizes (Fig. 6) as reported earlier in Egyptian Water buffalo (Roshdy and Derbalah, 2017), one humped camel (Roshdy, 2009) and pig (Ceylan and Alabay, 2017). TEM images reveal the presence of numerous interdigitating processes on the surface of TECs. This was in accordance with the observation reported earlier in buffalo (Rammaya *et al*., 2011). TEM studies showed degenerative nuclear and cytoplasmic structure in the central part of Hassall's corpuscles, which were involved in thymic

involution as reported earlier in Egyptian Water buffalo (Roshdy and Derbalah, 2017), one humped camel (Roshdy, 2009), Nandanam chicken (Kannan *et. al*., 2015) and camel (Aly *et al*., 1988). Additionally, TEM provides insights into the ultrastructure of other cell types within the thymus, including macrophages and mast cells. Similar finding were observed in goat (Sivagnanam *et al*., 2017). The macrophage was large, irregular in shape with a large nucleus and long, narrow and irregular cytoplasmic extensions (Fig. 7). The cytoplasm contains mitochondria, golgi apparatus, pleomorphic, dense bodies, which were intimately involved in the process of phagocytosis. The mast cell was characterized by the round nucleus showing chromatin clumping and cytoplasm was filled with numerous dense granules (Fig. 8). The membrane enclosed granules vary in electron density, shape, and internal structure. Few cytoplasmic organelles were also seen in the cell.

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