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ANATOMY AND MICROSCOPICAL STUDIES ON THYMUS OF A LARVIVOROUS FISH [*APLOCHEILUS PANCHAX* (HAMILTON, 1822)]

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ABSTRACT
The macro and microanatomy of thymus gland in *Aplocheilus panchax* (Hamilton, 1822) [a larvivorous fish] belonging to the Order: Cyprinidontiformes has been studied under light microscope (LM), scanning and transmission electron microscope (SEM + TEM) respectively. *A. panchax* possess apparently triangular shaped thymus gland which is located within the branchial cavity at the base of third and fourth gill arches. The surface structure of thymus gland shows numerous pores of varying diameter (0.75µm. to 1.5µm.) under scanning electron microscope (SEM). Microanatomically, the thymus is not well demarcated into cortex and medulla. This gland is composed of different types of lymphoid and non- lymphoid cellular components. The thymic epithelial cells and thymic trabeculae are also identified. The heterogeneity of thymic cells is need to further study for exploring the multifunctional aspects of this concerned lymphoid organ as a whole.

KEYWORDS: *A. panchax*, thymus, trabeculae, macrophage, *etc.*

INTRODUCTION

Thymus is a primary lymphoid organ of fish (Bowden *et al.*, 2005). This gland plays a crucial role in development of functional immune system and acts as an important site for T-cell development (Nakanishi, 1986; Tatner, 1996). The gross anatomy of this gland in teleosts was first studied by Stannius (1850) in angler fish (*Lophius piscatorius*). Generally this gland is situated superficially in contact with the epithelium of the branchial cavity (Fänge and Pulsford, 1985). The thymus gland is surrounded by a capsule (Chilmonczyk, 1992). This gland is composed of different types of lymphoid cells within a network of epithelial cells (Manning, 1994). However, the cortex and medulla are the most notable parts of thymus but hardly demarcated in teleosts (Trede *et al.*, 1998). Apart from that, the structural details of thymus gland in most of the Indian teleosts are still less studied so far. Larvivorous fishes are the most significant teleostean group that can control population of mosquito larva in urban and periurban areas of developing countries (Talwar and Jhingran, 1991; Chandra *et al.*, 2008). In India, *Aplocheilus panchax* (Hamilton, 1822) [Order: Cyprinidontiformes] is commonly used as a potential bio-controlling agent for mosquito larvae (Kumar *et al.*, 1998). This freshwater species mostly consume substantial amount of mosquito larva from variable environmental conditions of sewage drain water, tap water, *etc.* (Manna *et al.*, 2011). This study emphasizes on the microscopical details on thymus gland in *A. panchax* inhabiting sewage drain water to highlights its structural components as well as their probable functions in comparison with other freshwater teleosts.

MATERIALS & METHODS

i) Experimental specimen

Aplocheilus panchax (Hamilton, 1822) [Order-Cyprinidontiformes and Family-Aplocheilidae] is an indigenous larvivorous fish of South East Asia (fig 1). This species is widely distributed in all over South-East Asia. According to the *International Union for Conservation of Nature (*IUCN), there are no known threats to the population of this species (*i.e.*, 'Least Concern').

ii) Anatomy

For anatomical studies, live and healthy *A. panchax* specimens were directly collected from the various ponds and sewage drain water of different area of Howrah and East Midnapore districts of West Bengal, India. The collected specimens were brought to the laboratory and acclimatized with the laboratory conditions [temperature: 20˚ to 25˚C, humidity: >40%] for 24 hours. The specimens having total body length (3 - 6) cm. and body weight (5 - 6) gm were sorted out and anaesthetized by using MS–222 (dose: 100mg/L – 200mg/L). The thymus glands of *A. panchax* were dissected out from the opercular cavity and immediately fixed in aqueous Bouin's fluid [75 ml of saturated aqueous picric acid solution is added with 25ml of 40% formaldehyde and 5 ml of glacial acetic acid is mixed to make fresh aqueous Bouin's fluid] for overnight. The glands were mounted by glycerine on grease-free glass slides and examined under light microscope (LM).

iii) Microanatomy

For microanatomical study, the procured thymus glands of *A. panchax* were separately fixed in aqueous Bouin's solution for (6-10) hours at room temperature (25˚C - 30˚C). The tissues were then washed and subsequently

dehydrated through graded ethanol. The dehydrated tissues were immediately transferred into Cedar Wood Oil for (24 –48) hours. The tissues were then cleared in xylene and embedded in graded paraffin - xylene mixture under a thermostat vacuum paraffin-embedding bath [temperature 58°C – 60°C] for a period of 60 minutes each respectively. The serial thin sections (about 5µm) of the thymus gland were cut by using rotary microtome and stretched on Mayer's albuminised glass slide. The sections were then stained with haematoxylin - eosin and examined under light microscope (LM).

iii) Scanning electron microscopical (SEM) study

For scanning electron microscopical (SEM) study, the thymus gland of *A. panchax* specimens were fixed in 2.5% glutaraldehyde in 0.1 (M) phosphate buffer (pH. 7.2 – 7.4) at 4° C for 1 –2 hours. After completion of the fixation, the samples were then rinsed in same buffer *i.e.*, 0.1 (M) phosphate buffer (pH 7.2 – 7.4) for 3 changes at a regular interval of 15 minutes and dehydrated in graded chilled acetone. The dehydrated specimens were critically dried (CPD) with liquid carbon dioxide (CO_2) and then carefully placed on aluminium stub and coated with gold (thickness: 20nm–30nm). The prepared samples were examined under scanning electron microscope (SEM: LEO - 435) at 0° tilt angle, operated at 15 – 20 kV.

iv) Transmission electron microscopical (TEM) study

For transmission electron microscopical study, the thymus glands of *A. panchax* were separately dissected out and immediately fixed in 2.5% glutaraldehyde in 0.1(M) phosphate buffer (pH 7.2–7.4) at 4°C for 1– 2 hours. After completion of the primary fixation, the tissues were rinsed in the same buffer and further fixed in 1% osmium tetraoxide (OsO_4) in 0.1 (M) phosphate buffer (pH 7.2–7.4) for 1hour at 4°C. The thymus glands were then dehydrated in graded, chilled acetone (at 4°C). The dehydrated tissues were embedded in araldite mixture and incubated at 60° C for 48–72hours. Ultrathin sections (70nm –90nm) were cut with the help of ultramicrotome (Leica Ultracut UC6) and collected on copper grids; stained with uranyl acetate solution and lead citrate; examined under transmission electron microscope [TEM: MORGAGNI – 268D] operated at 40kV.

RESULTS

Anatomically, the thymus gland of *A. panchax* is bilaterally placed and apparently triangular in shape, observed under optical light microscope (LM). Microanatomically, this gland is located at the uppermost part of the mid dorso-lateral region on the either side of opercular cavity at the base of third and fourth gill arches (Fig. 2) which is also very close to the head kidney (Fig. 3). The thymus gland is hardly characterized into cortex and medulla. A large number of lymphoid cells are frequently noted within the thymic stroma of *A. panchax* (Fig. 4). Under scanning electron microscope (SEM), the thymus gland in *A. panchax* also shows clusters of cells and mucous secretory pores with variable diameter ranging from 0.75µm to 1.5µm. (Fig.5). The polygonal microridges (thickness: 0.2µm) are also well demarcated on the surface of the said tissue (Fig. 5). Under transmission electron microscope (TEM), the thymo pharyngeal epithelial cells are frequently observed at cortical region of thymus (Fig. 6). This cell possess prominent chromatinized nucleus. Heterochromatin components are evenly distributed within the peripheral part of the nucleoplasm (Fig. 6). Thymocytes and thymoblasts are distributed in throughout the thymic stroma (Fig. 7). Thymocytes are showing chromatinized nucleus with distinct nucleolus at the central part of the cytoplasm (Fig. 7). This cell is also observed within the thymic trabeculae (Fig. 7). The shape, size and diameters of trabeculae are variable within the thymic tissue. The basement membrane, blood capillaries, peri-trabecular epithelial cells, endothelial cells and connective tissue are the key component of thymic trabeculae (Fig. 7). Apart from that, free granulocytes and macrophages are also distinctly identified within the trabeculae (Fig. 7).

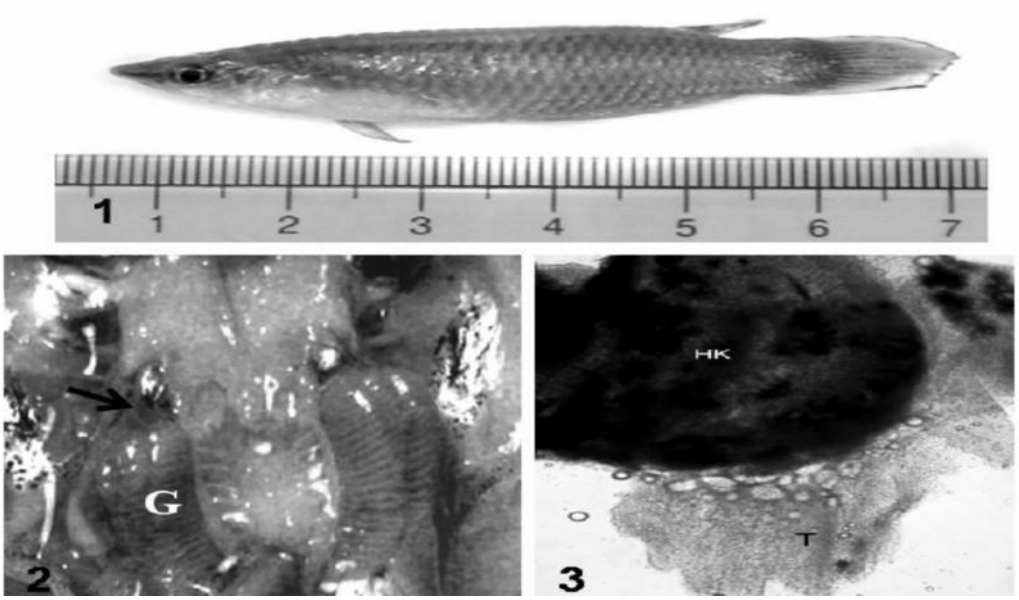

FIGURE 1: *Aplocheilus panchax* (Hamilton, 1822); a teleostean larvivorous fish of South East Asia.
FIGURE 2: The photograph indicates true position of thymus gland (→) at the base of gill arches (G). [Not to Scale]

FIGURE 3: The anatomical photomicrograph (from temporary preparation) shows that triangular thymus gland (T) of *A. panchax* is closely associated with head kidney (HK). [Mag: x 100 (approx.)]

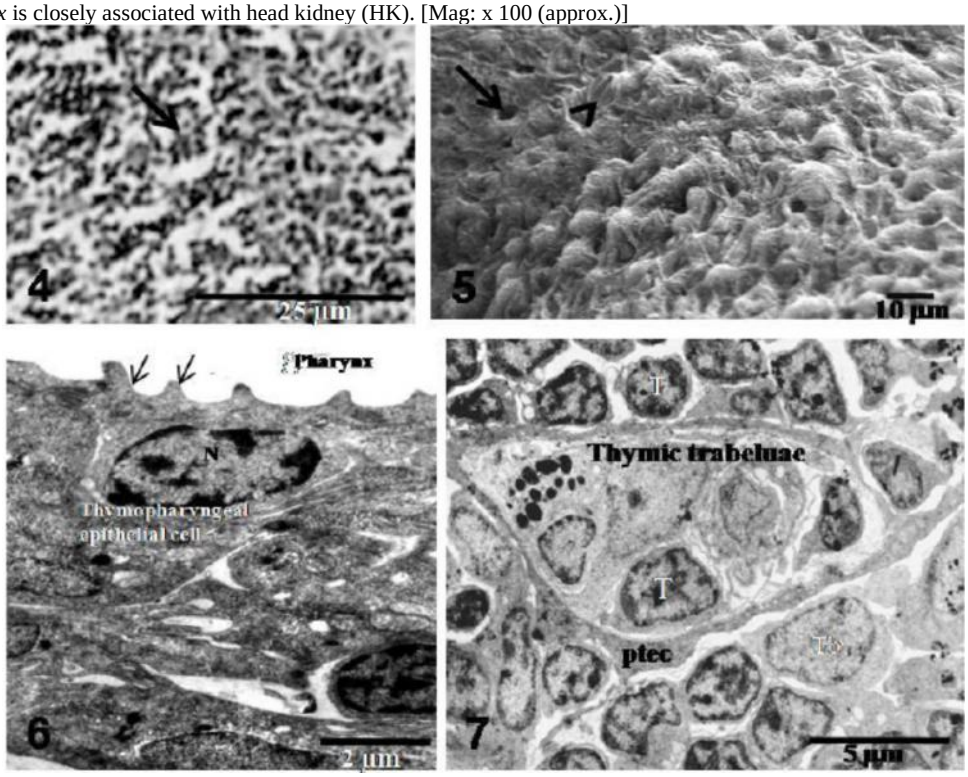

FIGURE 4: The microanatomy (from paraffin sections) of thymus gland shows large number of thymocytes within the stroma.
FIGURE 5: The mucous secretory pores (→) and polygonal microridges (>) are distinctly identified at the surface of thymus gland of *A. panchax*.
FIGURE 6: The transmission electron micrograph shows prominent thymopharyngeal epithelial cell within thymus stroma of *A. panchax* which is located very close to the pharynx. This cell shows broad apical part with several microvilli (arrows). The spherical, chromatinized nucleus (N) is also noted at the central part of this epithelial cell.
FIGURE 7: The thymocyte (T) is distinctly marked within the thymic trabeculae. Granulocyte, macrophage, *etc.* are also noted. The peritrabecular epithelial cell (ptec), thymocytes (T) and thymoblast cell (Tb) are also observed at the peripheral part of thymic trabeculae.

DISCUSSION

The present study represents the macro- and microanatomy of thymus gland in larvivorous teleost (*A. panchax*) inhabits in different freshwater bodies. This species can tolerate a wide range of polluted environmental conditions (Talwar and Jhingran, 1991). The thymus gland in fish is commonly considered as 'being well protected against antigenic stimulation' (Chilmonczyk, 1985). The presence of numerous pores on the surface of thymus gland may facilitate the passage of bioactive compounds from the external environment (Chilmonczyk, 1985). Therefore, the specific cell mediated protective measures of thymus gland against invading pathogens in this fish would be an interesting question to explore. Apart from their variability in occurrences of different environmental conditions, the structural as well as cellular organization of thymus gland in *A. panchax* shows remarkable similarities with other telelostean species. This gland may acts as a major site for differentiation, maturation and storage of T-cell (Manning, 1994). It is also regarded as the first organ that acquires lymphocytes during histogenesis of lymphoid tissue (Zapata *et al.*, 1996). The differentiating lymphoid cells and epithelial cells within the stroma are generally organized in cortical and medullary zones of thymus gland (Manning, 1994). This type of demarcation is noted under light microscopical studies (Zapata *et al.*, 1996) but it is not very distinct in fish including *A. panchax*. The presence of lymphoid cells within the epithelial framework is an indicative of interaction between ectoderm and endoderm at the time of development (Schuurman *et al.*, 1997). The thymic epithelial cells are one of the most important cellular components that are significantly involved in development of thymocyte through intercellular communication (Sun *et al.*, 2013). The other thymic components *i.e.*, the capsule, trabeculae and perivascular components, *etc.* are believed to be mesenchymal in origin (Schuurman *et al.*, 1997). The arrangement of trabeculae in higher vertebrates is also very unique which extends from cortex to medulla of thymic stroma (Bowden *et al.*, 2005). This structure in *A. panchax*, is extending towards the interior part of the organ and subdivided the tissue into an extensive interconnecting space. Several authors have reported that, the thymic trabeculae are significantly acts as a 'blood-thymic barrier' which may regulate the exchange of substances between circulatory system and thymus (Ranga *et al.*, 1982; Henry *et al.*, 1992; De and Pal, 2000). In consequence to that, the cellular association within and peripheral part of thymic trabeculae may denote as an important cytological feature regarding development of

thymocytes. Thus, an extensive study on the basis of the present base line data is needed to explore multiple aspects of thymic biology in this experimental species (*A. panchax*).

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