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Phagocytic Interaction Within the Olfactory Neuroepithelium of Fish (*Labeo rohita*) with the Functional Association of Rodlet Cells

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Abstract

The phagocytic macrophages within the olfactory neuroepithelium of fish [*Labeo rohita* (Hamilton, 1822)] are examined under light and transmission electron microscope. The distribution of macrophages inside the olfactory neuroepithelium of fish is determined by the distinct morpho-functional characteristics of each cell. The rodlet cells are different due to their potential functional structures and level of maturity. The macrophages, mast cells, and rodlet cells are jointly responsible for the elimination of the pathogenic load from the olfactory neuroepithelium of fish, i.e., cell-mediated defense (non-specific interaction). This notable feature within the olfactory neuroepithelium is characteristically indicated as the part of neurological defense against invasive pathogens.

***Key words*:** *Labeo rohita,* Neuroepithelium, Pathogens, Phagocytic, Macrophage, Rodlet cell

Olfaction is a special type of chemical sense that is involved in the recognition and discrimination of chemical cues from the external environment [1]. This process plays an important role in the defense against the pathogen, procurement of food, recognition of sex, species identification, defense against predators, parental care, migration, etc. [2]. The olfactory apparatus of fish generally consists of the anterior nasal opening (ANO), posterior nasal opening (PNO) that separates the nasal flap, olfactory chambers, olfactory bulb, olfactory nerve tracts, and an olfactory lobe of the brain [3]. The Olfactory neuroepithelium of fish is a specialized cellular integration that requires protection. The olfactory neuroepithelium is a pseudostratified structure. The cellular interaction between vascular elements / or a neural phagocytic cells and neural cells [4] / or neuroglia cells actively participated in neuroprotective function within the olfactory neuroepithelium of fish. But such type of neural action is hardly characterized in detail, within the science of chemosensory biology. The presence of specialized cells i.e., goblet cells, rodlet cells, mast cells, heterophilic granulocyte cells, macrophage, plasma cells, etc. are responsible directly or indirectly for the protection of olfactory neuroepithelium of fish, as well as against invasive pathogens from the outer environment [5]. Especially the cell viz., macrophages have strong phagocytic properties (i.e., scavenging action) within the olfactory neuroepithelium of fish. Although the morphs of macrophages show different structural peculiarities in connection to their diverse functions. Apart from that the association of goblet cells, rodlet cells, and mast cells is very much important to elaborate the neuro-immunological duplex network to protect the olfactory neuroepithelium of the very species. This type of special activity not only protects the olfactory tissue integration but also protects the invasive pathogens and or the pathogenic biomolecules present in the aquatic system.

MATERIALS AND METHODS

Macroanatomy study

Labeo rohita is a common Indian Major Carp of Southeast Asia, belonging to the order of Cypriniforms. These species are considered as 'Least Concern' according to IUCN Red List Category Ver. 3.1. Live, adult (250±5) gm., sex-independent *Labeo rohita* were collected from the freshwater pond at Midnapore town, West Bengal, India. For 24 hours, the species were acclimatized in laboratory conditions at room temperature. The fish were anesthetized with MS-222 dose: 100mg/L^{-1} - 200mg/L^{-1} [6], and the olfactory organ was dissected out from the dorsal side of the head. The macro-anatomical structure was examined under the stereo zoom light microscope [ZEISS: Stemi doc 508].

Microanatomy and transmission electron microscope (TEM) study

Olfactory organs of *Labeo rohita* are immediately fixed with Karnovsky's fixative [2.5% glutaraldehyde and 2% paraformaldehyde with 0.1M PB (phosphate buffer pH- 7.4)] for 24 hours at 4°C. Wash in Phosphate buffer (thrice, 15 minutes each). Secondary fixation was performed by 1% osmium tetraoxide (OSO_4) [dissolved in PB 0.1 M, pH- 7.4 for 1 hour at room temperature]. The post-fixed tissues were dehydrated with progressively higher concentrations of cold acetone from 30% to 100% during the stipulated time. After the block preparation, the same tissue was processed for

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microanatomy (stain by 1% toluidine blue) and examined under Axio Vision LE (version 4.3.0.101) [Carl Zeiss Vision, GmbH, Germany]. The ultrathin sections (50 nm-70 nm) were stained by 1% uranyl acetate and 1% lead citrate respectively and viewed under HR- TEM [TALOS: THERMO SCIENTIFIC at 200 kV].

Scanning electron microscope (SEM) study

Labeo rohita dorsal head region was dissected to expose the external nostril and rosette. Tissue was immediately fixed in 2.5% glutaraldehyde solution (Sigma-Aldrich, EM Grade) in 0.1 M phosphate buffer or PB [Na_2HPO_4 and NaH_2PO_4; pH -7.4] for 4 hours at 4°C. After the fixation process was completed then the olfactory tissue was rinsed three times at 15-minute intervals in phosphate buffer (0.1 M, pH -7.4). The tissue sample was fully dehydrated using ascending chilled acetone (30% - 50% - 70% - 90% - 100%) with an interval of 15 minutes each and then in 100% acetone over 30 minutes followed by isoamyl acetate. Afterward, the tissue conducted critical point drying (CPD) using liquid carbon dioxide in a critical point drier (Hitachi 8CP2). The dried tissue was mounted on a metal stub, coated with gold (16nm thick) using a sputter coater (Quorum Q150TES), and viewed under a scanning electron microscope (SEM, Zeiss EVO18) operating at 20 kV [7-8].

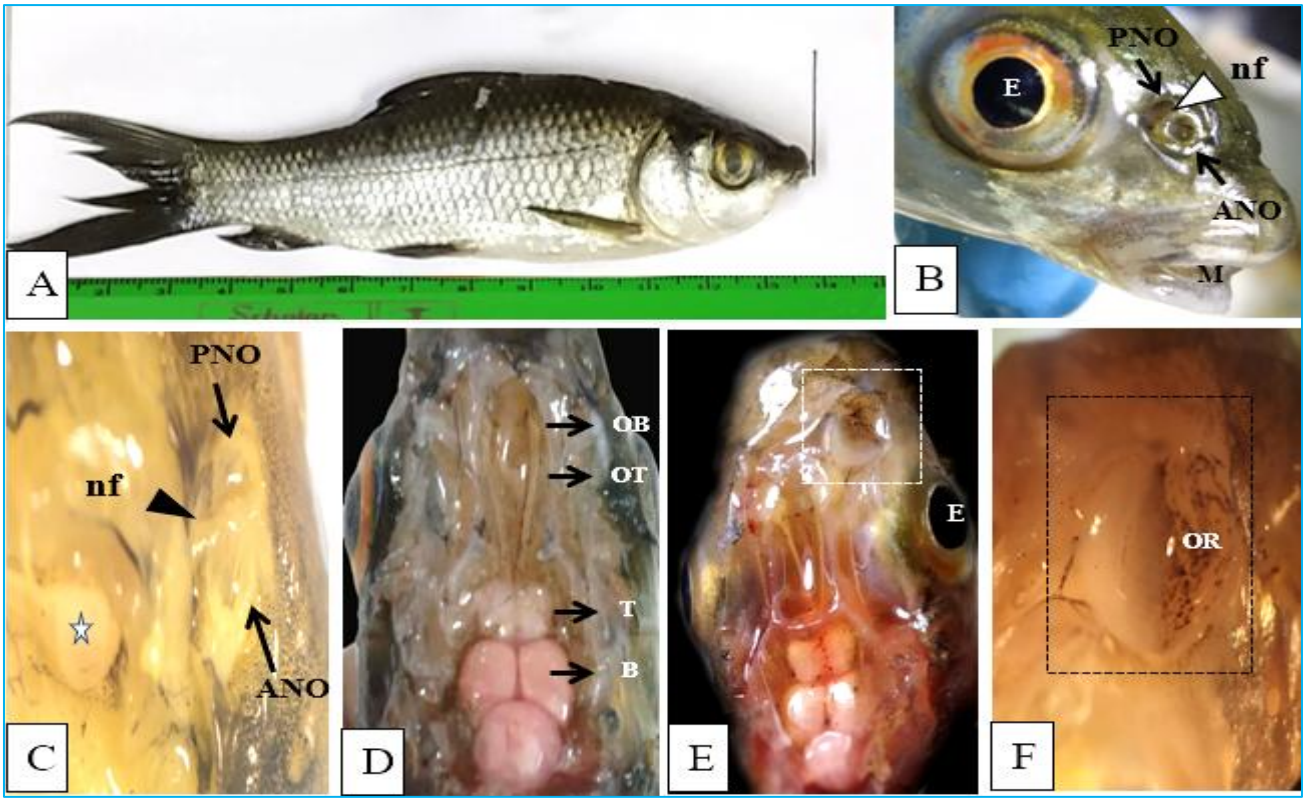

Fig 1 [**A**] Photographs shows the morphoanatomy of model species of *Labeo rohita* (Hamilton, 1822). [**B** and **C**] The anterior nasal opening (ANO) and posterior nasal opening (PNO) are differentiate by nasal flap (nf) at the anterior part of the head of *Labeo rohita* [Not to scale]. [**D** and **E**] The telencephalon (T) of the brain (B) is a directly communicated to olfactory bulb (OB) through the olfactory nerve (ON) of *L. rohita* [Not to scale]. [**F**] Photographs shows crescent shaped olfactory rosette (OR) behind the nasal opening, within the olfactory chamber of *L. rohita* [Not to scale]

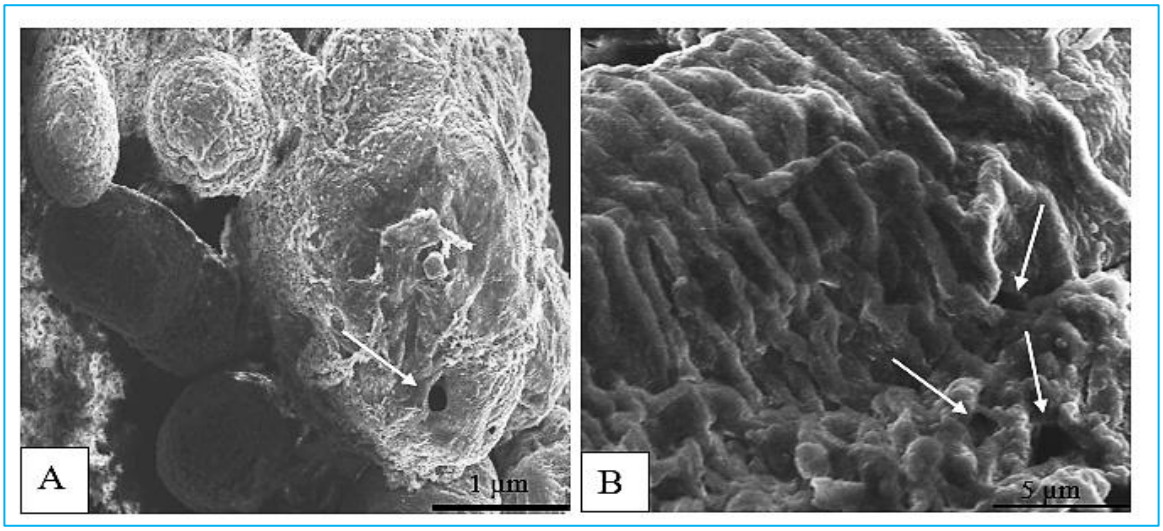

Fig 2 [**A**] The surface topography of olfactory lamellae under scanning electron microscope
[**B**] Electron micrograph indicates definite mucus secreting pores (solid arrow), the space for the cellular communication in between olfactory neuroepithelium and external aquatic environment

RESULTS AND DISCUSSION

Olfactory neuroepithelium is the major part of olfactory lamellae and this structure is associated with olfactory raphe (Fig 3A). Olfactory rosette is placed within the olfactory chamber of the nasal sac of *Labeo rohita* (Fig 1 E-F). The specialized structure i.e., olfactory neuroepithelium shows goblet cells, sensory cells, non-sensory cells, basal cells, and vascular elements (Fig 3C). Goblet mucus secreting pore is clearly distinguished within the olfactory neuroepithelium (Fig 2 A-B). The morphos of macrophages within the olfactory neuroepithelium shows a prominent chromatinised nucleus, having pseudopodial structures and asymmetrical distribution of the protoplasmic organelles (Fig 4A). These macrophages are distributed in the different portions of the olfactory neuroepithelium of fish (Fig 4 B-E). The macrophage cell is very much affectionate to mast cells and rodlet cells respectively (Fig 4D). Mast cells are very prominent and associated with macrophages (Fig 4C). The rodlet cell shows different phases of maturity (viz., early, mature, and late stages) (Fig 3D). Macrophages are very much active within the olfactory neuroepithelium when they are very close to rodlet cells (Fig 4 B-E).The frequency of rodlet cells and macrophages is comparatively high at the upper zone of the olfactory neuroepithelium (Fig 4A). We observed macrophage is heavily loaded with sub cellular material and cellular debris (Fig 5C).

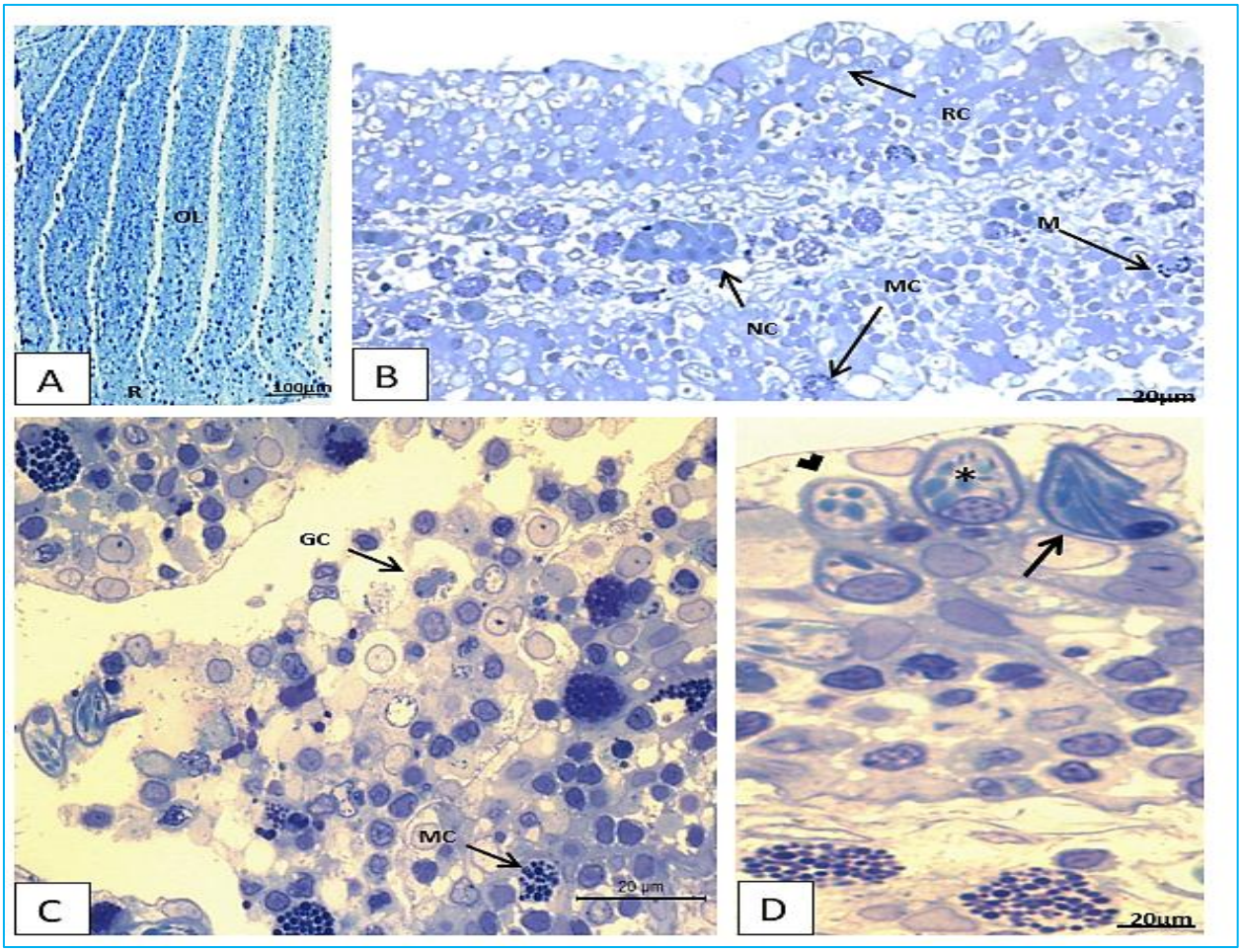

Fig 3 [**A**] Within the olfactory structure of *L rohita* the Olfactory lamellae (OL) are closely attached with the raphe (R) [**B**] Toluidine Blue stained section of Olfactory neuroepithelium shows the neural cell, Rodlet cell (R), Macrophages (M) nurse cells (NC) and Mast cells (M). [**C**] The frequent appearance of mast cells (MC) and goblet cells (GC) at the anterior portion of olfactory lamellae within the olfactory neuroepithelium of *L. rohita* [**D**] Different phase of Rodlet cells [i.e., early (arrowhead), intermediate (asterisk), and late (solid arrow)] are clearly identify at the anterior proximity within the olfactory neuroepithelium of *L. rohita*

Macrophages are recognized as phagocytic cells and have the ability for general defense within the olfactory neuroepithelium of fish [9]. The Lysosomal enzymes come into contact with intercellular pathogens and destroy the targeted biomaterials with their chronological interactions in a particular state of the situation demand [10]. Mast cells is a special kind of cell that also trigger the function of macrophages in a definite situation within the olfactory neuroepithelium and strengthen the neuro immunological activity at the time of cellular interaction [11]. The rodlet cell is thought to be a migratory blood cell derived from the circulating stem cell [12]. The rodlet cells and macrophages frequently appear within the olfactory neuroepithelium as responses to various pathogens within the nasal cavity of fish. The mature rodlet cell's surface region is covered in glycocalyx which may shield the cell from physical or chemical harm and keep other cells apart to avoid undesirable stress [13]. The breakdown of cytosolic and sub-cellular elements may be a sign that the degenerating rodlet cell has a holocrine way of secretion [14]. It is assumed that the rodlet cell participates in a "nonspecific immune response" via the holocrine way of secretion to defend the olfactory neuroepithelium from invasive pathogens present in the nasal cavity, even though the biochemical nature of the secreting substances of degenerative rodlet cells has not yet been investigated. It is also known to us that the chemosensory system is highly protective and generously nurtured by its own set of sub-cellular interactions that are monitored by its homeostatic mechanisms. The activated macrophage functions in different way to protect the neural tissue at a particular time and in a particular ecological habitat.

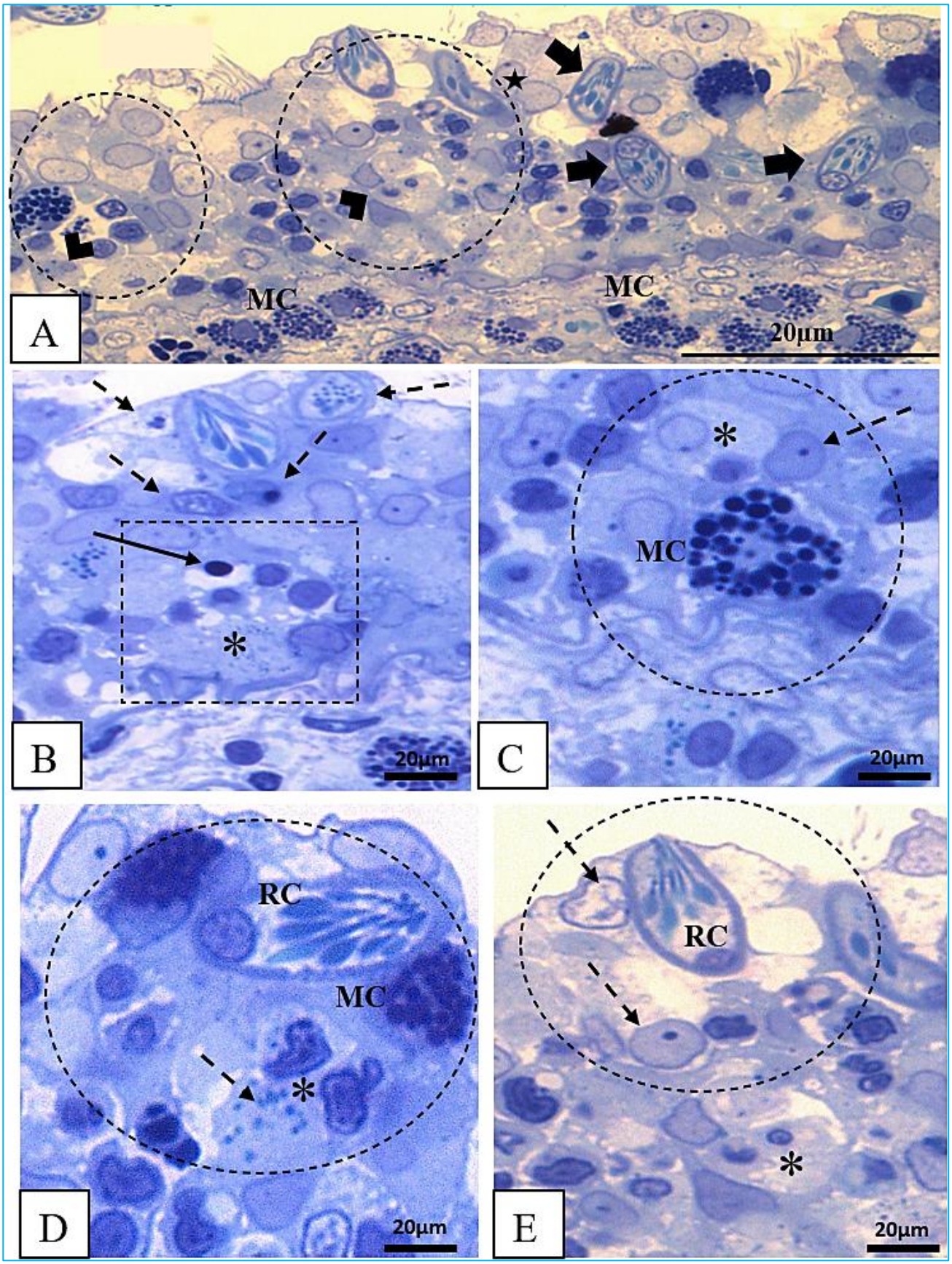

Fig 4 [**A**] Light microscope (LM) photograph shows the olfactory neuroepithelium of *Labeo rohita* with distinct appearance of non-neuronal vascular elements i.e., Morphs of active Macrophages (arrow head) [marked area], Mast cells (MC) and Different stages of Rodlet cells (solid arrow) are well scatter as per their functional needs. [**B**] Pathogenic substances (broken arrows) are identified within the olfactory neuroepithelium of *L. rohita* and these pathogenic substances (solid arrow) are also engulfed by phagocytic macrophage (asterisk) [rectangular marked area]. [**C**] Light microscopic observation indicates phagocytic macrophage (asterisk) is closely associated with Mast cell (MC) within the olfactory neuroepithelium of *L. rohita* (circular marked area). [**D**] During the time of phagocytic Macrophage (asterisk) and Mast cell (MC) interactions, the close the presence of Rodlet cell (RC) is also evident under light microscope (circular marked area). [**E**] This LM picture focus on Rodlet cell (RC) that is distinctly affectionate to pathogenic substance (broken arrows) within the olfactory neuroepithelium of *L. rohita* (circular marked area)

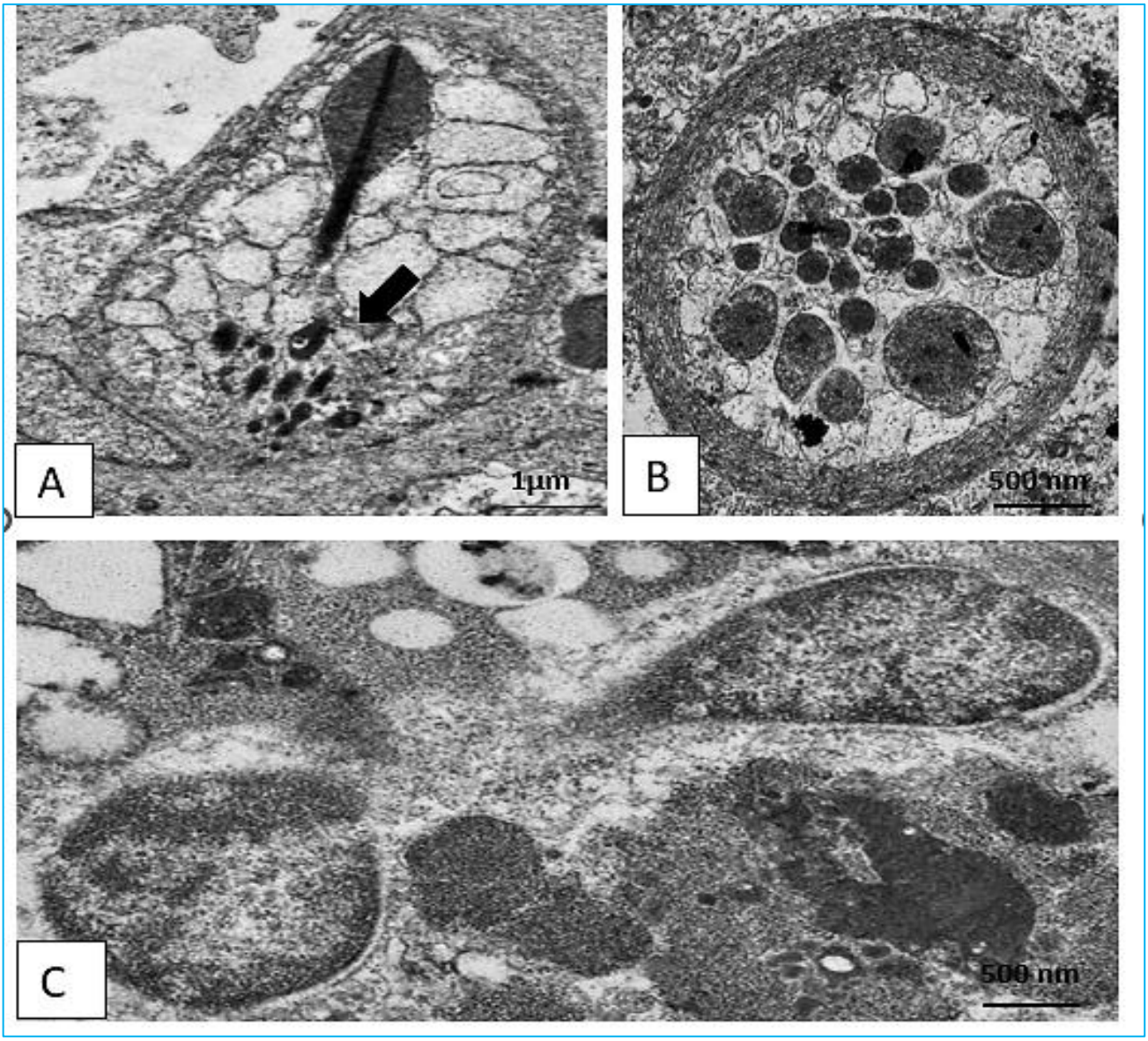

Fig 5 [**A**] Electron micrograph shows the mature rodlet cell containing secretary granules with fibrous exoplasm (solid arrow) [**B**] The EM photographs show degenerative rodlet cells after the holocrine secretion from the olfactory neuroepithelium of *L. rohita* [**C**] Electron micrograph shows the dilatated subcellular components within the olfactory neuroepithelium of *L. rohita*

CONCLUSION

The olfactory neuroepithelium of fish is directly exposed to the aquatic environment and needs definite neural protection against hostile living biomolecules and pathogens. Phagocytic macrophages in association with neuroprotective cells (viz., goblet cell, rodlet cell, mast cell, macrophage, etc.) trigger the said process within the olfactory neuroepithelium of fish. Specially the Rodlet cells helps the Macrophages and Mast cells to eradicate the pathogens through phagocytic interaction within the olfactory neuroepithelium of fish. This pertinent event focuses the outcome of the immune-neurological duplex networking system of the species concerned.

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