Indian J. Applied & Pure Bio. Vol. 39(2), 665-670 (2024). **ISSN: 0970-2091**
A Web of Science Journal

A mechanistic insight into the cytomorphology of liver macrophages in tilapia (*Oreochromis* sp.)

***Srikanta Guria and Rahit Ali**

Post Graduate Department of Zoology, Barasat Govt. College, Barasat, Kolkata-700124, West Bengal (India)
***Corresponding author**: Dr. Srikanta Guria, Assistant Professor, Post Graduate Department of Zoology, Barasat Govt. College, Kolkata-700124, West Bengal (India)
E-mail: guriasrikanta@gmail.com, srikantaguria81@gmail.com

Abstract

Macrophages are found primarily in the fish spleen and kidney, and to a lesser extent in liver. They are key cells for dealing with foreign particles. Liver is a target tissue for lesions related to chemical pollution. However, there is limiting literature available on the macrophages within the liver in fishes. Melano-macrophage centres (MMC), are normally located in the stroma of the tissue of the spleen, liver and the kidney. Melano-macrophage centres increase in size or frequency during environmental stress and chemical pollution. We aimed to characterize the morphological and functional aspects of liver-associated macrophages in tilapia. Tissues from liver were removed from tilapia (*Oreochromis* sp.) and mashed in (0·1) M phosphate buffer saline (pH 7·2) in presence of trypsin-EDTA. From cell suspension, adherent macrophages on glass slides were analyzed. Kidney and Liver tissues were processed for routine histological examination. Liver macrophages (LM) showed the property of phagocytosis. Tendency of cell fusion was noticed. Macrophage aggregates on glass slides and tissue sections were poorly, irregularly organized. Compared with the liver, macrophages (MACs) accumulations in the kidney tissues were larger in size and distinct. Hepatic alterations and the presence of large MMCs in the livers and necrotic macrophages/ immune cell agglomerate of fishes may be related to the high levels of heavy metal contamination and pollution.

Key words : Liver, Macrophages, Macrophage Aggregates (MA), Melano-macrophage centres (MMC), Chemical Pollutants.

Macrophages are chief cells of the reticuloendothelial system, being present primarily in the fish spleen and kidney, and to a lesser extent in liver. They are key cells for dealing with foreign particles and cellular debris[1,3,4].

Liver is a target tissue for lesions related to waste water-related contaminants and

chemical pollution. The presence of liver macrophages within mammalian species, like human and rat/mice has been well studied. However, there is limiting literature available on the macrophages within the liver in fishes. Macrophages in tissues can increase in number not only with toxicant/ chemical pollutants exposure but also with other non-toxicant related conditions, such as nutritional imbalances and age[1,2,3]. Melano-macrophage centres (MMC), also called as macrophage aggregates (MA), are distinctive groupings of pigment-containing cells (including melanins, lipofuscin/ ceroid, and hemosiderin pigments) within the tissues[2]. In fish they are normally located in the stroma of the tissue of the spleen, liver and the kidney[2].

Melano-macrophage centres increase in size or frequency during environmental stress and have been suggested as suitable biomarkers for water quality in terms of chemical pollution[2].

Researchers showed numerous pigment cells as macrophages (MACs) were seen within the parenchyma of liver and spleen of Ohrid trout. They showed great similarities with the MACs found in other trout and other fish species also[3,4]. Previous researchers showed a significantly increased amount of pigmented MACs within the spleen and to a lesser extent in the liver, may be connected with aquatic pollution[2].

We aimed to characterize the morpho-logical and functional aspects of liver-associated macrophages in tilapia.

Alive fish samples of tilapia (n=9) were collected from local markets. Tissues from liver were removed from tilapia (*Oreochromis* sp.) and mashed in (0·1) M phosphate buffer saline (pH 7·2) in presence of trypsin- EDTA. From cell suspension, adherent macrophages on glass slides were stained by Giemsa and Neutral Red. Activated charcoal particles in normal saline (0·9% NaCl) was used for phagocytosis study. Kidney and Liver tissues were processed for routine histological examination and stained with hematoxylin and eosin.

Morpho-functional study :

We reported on the occurrence of macrophages within the tilapia liver exhibiting conventional ultrastructural features (*e.g.* presence of pseudopodia, extensive lysosomal apparatus). Result showed numerous macrophages as free or in aggregates (MAs) (Fig. 1). Tendency of cell fusion was noticed (Fig. 2). Macrophage aggregates were found on glass slides and tissue sections but were poorly, irregularly organized (Fig. 1, Fig. 2 and Fig. 4).

Neutral Red responses :

Macrophages showed neutral red positive reaction for lysosomal enzyme activity (Fig. 2).

Cell aggregation and pyknosis :

Different stages of aggregation in macrophages (MA) were noticed (Fig. 3). Some cell exhibited necrosis and pyknosis (Fig. 3).

Histological analysis of liver and kidney :

In the liver, numerous small accumulations of macrophages, were found between parenchymal cells and in the connective tissue of the stromal vascular-biliary tracts (Fig. 4A, B).

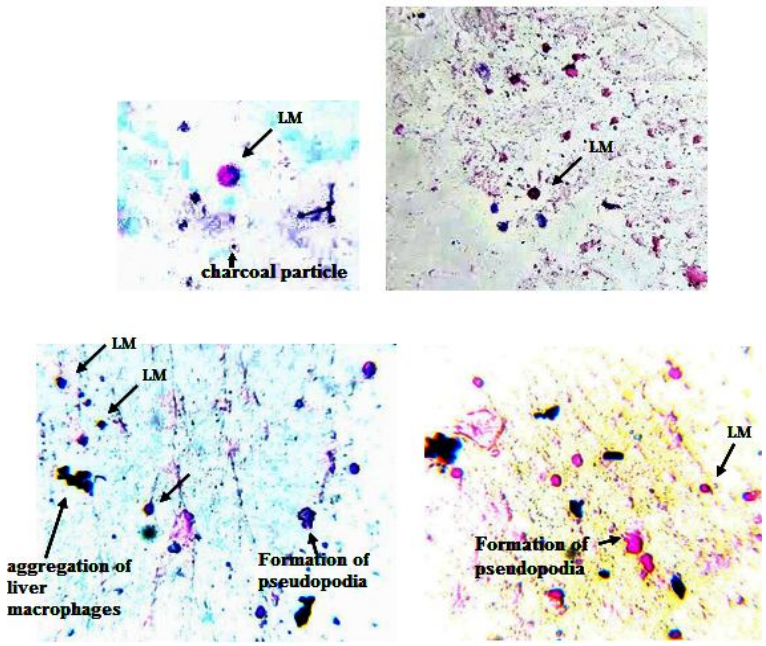

Fig. 1. Giemsa stained liver macrophages (LM) of tilapia showing their morphology on glass slides. Formation of pseudopodia, attachment of charcoal particles and aggregation in macrophages (MA) were noted (x400)

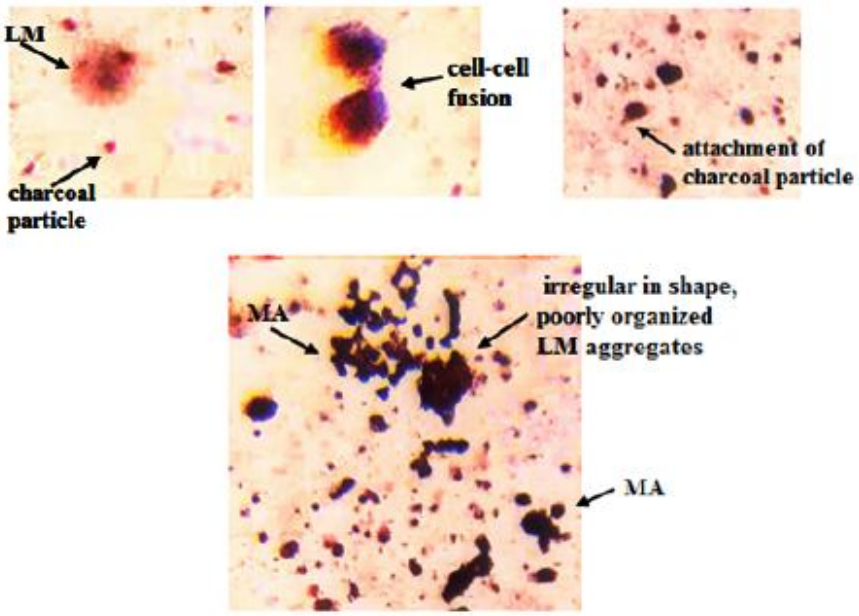

Fig. 2. Different stages of phagocytosis were observed in neutral red positive LM like attachment of charcoal particles, cell-cell fusion, and cell aggregation on glass slides [indicated by arrow (x400)]

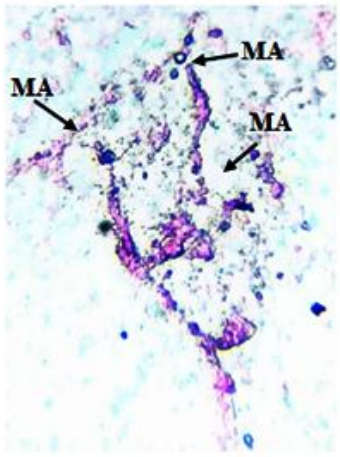

Fig. 3 (A)

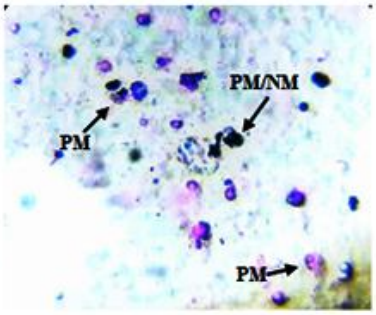

Fig. 3 (B)

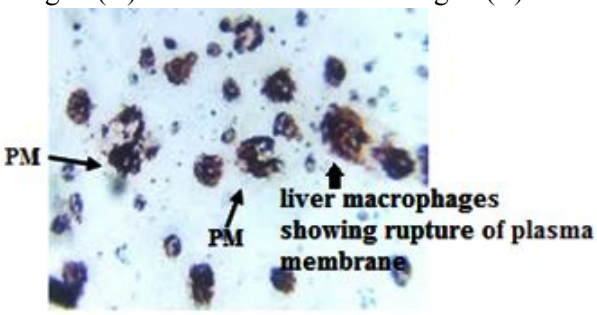

Fig. 3 (C)

Fig. 3(A). Giemsa stained stages of aggregation in LM in tilapia on glass slides (x100)
Fig. 3 (B). Some Giemsa stained liver macrophages showing rupture of plasma membrane (x400)
Fig. 3 (C). Some neutral red positive liver macrophages showing rupture of plasma membrane (x400)
Pyknotic macrophages (PM)/ Necrotic macrophages (NM) indicated by arrow

MACs in kidney tissues was also found to be scattered throughout the parenchyma. Compared with the liver, the MACs accumulations in the kidney tissues were larger in size and distinct. Extensive accumulations of MAs in kidney was noted (Fig. 4C).

Previous studies have shown an increase in number and size of macrophages and macrophage aggregates (MAs), Melano-Macrophage Centres (MMCs) in fish collected at contaminated sites[7]. Some researchers showed hepatic alterations and the presence of large MMCs in the livers and necrotic macrophages/ hepatocyte, immune cell agglomerate of fishes may be related to the high levels of heavy metal contamination (Cd and Cu) at the sampling sites[7]. Jaffal *et al*.,[7] analyzed cadmium (Cd) and copper (Cu) concentrations in the liv er of Kerguelen brown trout, and assessed the possible impacts of these metals on hepatic histopathology[7]. Clear

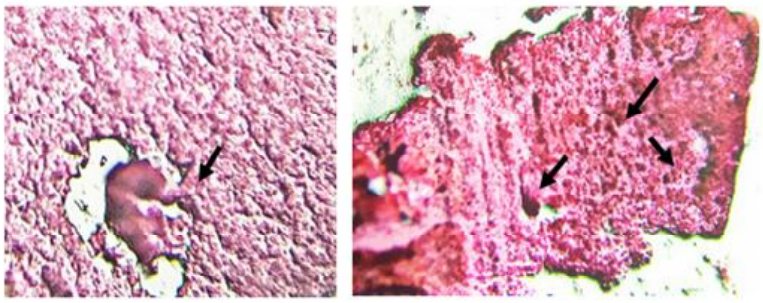

Fig. 4 (A). Histological sections of hematoxylin and eosin stained liver of tilapia. Note the macrophage accumulation/aggregation indicated by arrow (x400)

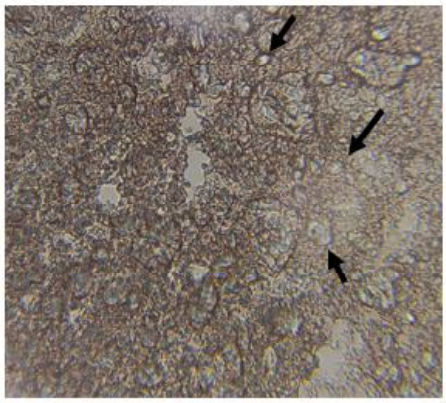

Fig. 4 (B). Histological section of PAS stained liver of tilapia. Note the macrophages indicated by arrow (x400)

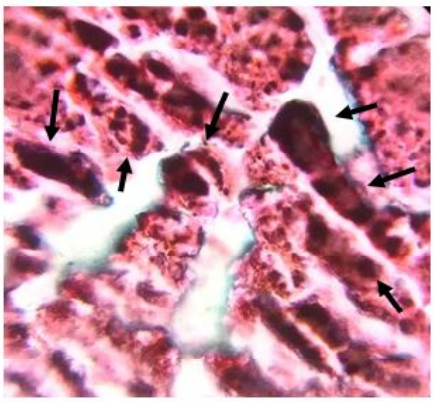

Fig. 4 (C). Histological section of hematoxylin and eosin stained kidney of tilapia. Note the macrophage accumulation/ aggregation/ Melano-Macrophage Centres (MMCs) indicated by arrow (x400)

hepatic disturbances (fibrosis, increased melanomacrophage centres [MMCs]) were observed in all tested trout due to anthropological reasons[7].

The wastewater treatment plants (WWTP) effluents and contaminants comprising metals, organic pollutants may affect macrophage aggregation in liver and fish health [8-10].

Wolke[11] stated macrophage aggregates (MAs), or melano-macrophage centers (MMC), are not confined to fish and have been observed in other poikilothermic vertebrates. The aggregations are most commonly found in the spleen, head kidney and liver, especially in relation to inflammation[11].

Present result showed numerous macrophages as free or in aggregates (MAs) (Fig. 1). LM showed the property of phagocytosis. Formation of pseudopodia, attachment of charcoal particle on surface, tendency of cell fusion was noticed (Fig. 2).

Result showed neutral red positive cells indicating the presence of lysosomal compartments confirming their phagocytic nature (Fig. 2). Macrophage aggregates on glass slides and tissue sections were poorly, irregularly organized (Fig. 1, Fig. 2 and Fig. 4). Extensive accumulations of MAs in kidney tissues was noted (Fig. 4).

Guria[5] isolated the macrophages from head kidney of tilapia and rohu to study morpho-functional alteration. Head kidney Macrophages (HKM) showed the property of phagocytosis and aggregation[5].

Guria *et al*.,[6] assessed inflammatory cells like macrophages in the exudate from swim bladder of rohu (*Labeo Rohita*). They also showed the property of phagocytosis and aggregation[6]. Our present result corroborated previous study.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors acknowledge to Head, Post Graduate Department of Zoology and Principal, Barasat Govt. College for necessary support.

References :

1. Agius, C. (1979) *J. Fish Diss*, *2:* 337-343.
2. Agius, C and R. J. Roberts (2003) *J. Fish Diss*, *26:* 499-509.
3. Blazer, V. S., D. E. Facey, J. W. Fournie, L. A. Coutrney and J. K Summers (1994) SOS Publications, Ed: Stolen, J.S., Fletcher, Fair Haven, New Jersey, (1): 169-185.
4. Evans, D. H. (1998) Ed: CRC marine science series Press. Inc. 2000 Corporate Blvd., N. W., Boca Raton Florida.
5. Guria, S. (2023) *Indian J. Applied & Pure Bio*, *38*(3): 1502-1509.
6. Guria, S., O. Middya, A. B. Hossain and D. Saha (2023) *Bull. Env.Pharmacol. Life Sci*, *12*(9): 48-53.
7. Jaffal A., B. Stephane, B.R. Sylvie, T. Alexandre, S. Wilfried and P.P. Severine (2015) *Polar Research* , *34:* 22784, http://dx.doi.org/10.3402/polar.v34.22784
8. Mehdi H., S.C. Lau, C. Synyshyn, M.G. Salena, E.S. McCallum, M.N. Muzzatti, J.E. Bowman, K. Mataya, L.M. Bragg, M.R. Servos, K.A. Kidd, G.R. Scott and S. Balshine (2021) *Sci Total Environ*, *759:* 143430. https://doi.org/10.1016/j.scitotenv.2020.143430
9. Restivo V.E., K.A. Kidd, M.G. Surette, M.R. Servos and J.Y. Wilson (2021) *Sci. Total Environ*, *10*(751): 141724. https://doi.org/10.1016/j.scitotenv.2020.141724
10. Tran, N.H., M. Reinhard and K.Y.H. Gin (2018) *Water Res*, *133:* 182–207.
11. Wolke, R.E. (1992) *Annual Review of Fish Diseases*, *2:* 91-108.