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ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of Inflammatory Cells in The Exudate Collected From Swim Bladder Of Rohu (*Labeo Rohita*)

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ABSTRACT
Swim bladders (SBs) represent a mucosal surface, that has role in immunity. In this study, we demonstrated inflammatory cells from SB responding to activated charcoal particles challenge. Inflammatory cells were collected from the swim bladder after 30 min of injection with charcoal particles from rohu (Labeo rohita). Smears were fixed and stained with Giemsa , leishman's eosin methylene blue solution, neutral red and NBT. Aggregation of macrophages and inflammatory cells, NBT positive cells and neutral red positive inflammatory macrophages were noticed. Teleost swim-bladder (SB) possesses mucosa-associated lymphoid tissue (MALT) and secretory IgT (sIgT). The presence of a great number of phagocytic cells suggests that fishes injected with charcoal particles showed increased acute inflammation which may be important for the organism's defense against infections. These parameters assessments may be important indicators of fish health status.
Keywords: *swim bladders, macrophages, inflammatory cells, inflammation, immunity.*

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INTRODUCTION

The air-filled organs (AOs) like lungs and swim bladders have evolved unique functions like air-breathing or buoyancy to adapt to different environmental conditions. Immune responses to foreign molecules in AOs have been described in the lungs of tetrapods. Similar to lungs, swim bladders (SBs) represent a mucosal surface, that has role in immunity. In this study, we demonstrated inflammatory cells from SB responding to activated charcoal particles challenge.

The swim bladder is a cavitary organ, with terminal circulation and facilities for inoculation and exudates collection for assessment of the cells accumulated due to inflammation **[1, 2, 3, 4, 5]**.The exudate is a mean for recognition of inflammatory process in tissues. In fish, the assessment of inflammatory response depends on identification of inflammatory cells like macrophages and granulocytes.

MATERIALS AND METHODS

Alive fish samples of rohu (*Labeo rohita*) were collected from local markets. To collect inflammatory cells, the fish were injected with 5ml of activated charcoal particles in normal saline (0·9% NaCl) into the swim bladder (Fig. 1). The swim bladder was washed with saline (0.9 gm% NaCl) and 0.09% EDTA, and the exudate was collected for studing cells from the inflammation **[3, 4, 5]**. Inflammatory cells were collected from the swim bladder after 30 min of injection with charcoal particles.

Smears were made on slides, air-dried, fixed and stained with Giemsa , leishman's eosin methylene blue solution, neutral red and NBT. From the smears the percentages of macrophages, lymphocytes, granulocytes were determined. Nitroblue tetrazolium (NBT) was used to detect the reactive oxygen species (ROS) in cells during respiratory burst. Morphology of Macrophages (M) and Granulocytes (G) were studied in the exudate.

Figure 1: Injection of charcoal particles in the swim bladder

RESULTS

In the exudates lymphocytes were seen as small cells. Macrophages were the largest cells observed in the exudates and exhibited cellular pleomorphism (Fig. 2 and 3). Granulocytes showed elliptical nuclei with

small cytoplasmic granules **[6]**. Aggregation of leishman's eosin methylene blue solution stained Macrophages (M) and Giemsa stained inflammatory cells were noticed (Fig. 4 and 5). Nitroblue Tetrazolium (NBT) positive cells which indicated reactive oxygen species (ROS) presented in the exudate (Fig. 6). Neutral red positive Inflammatory Macrophages (M) indicated presence of lysosomal enzyme (Fig. 7).

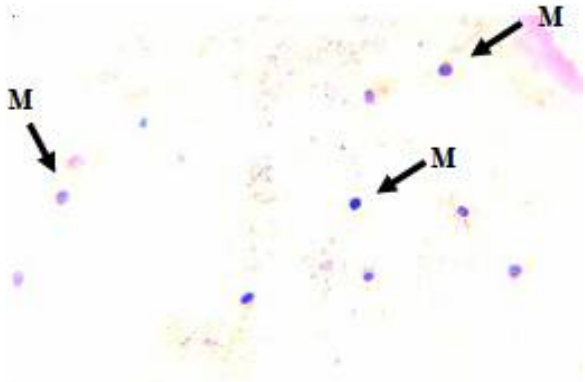

Figure 2: Giemsa stained Macrophages (M) presented in the exudate collected from swim-bladder (x100)

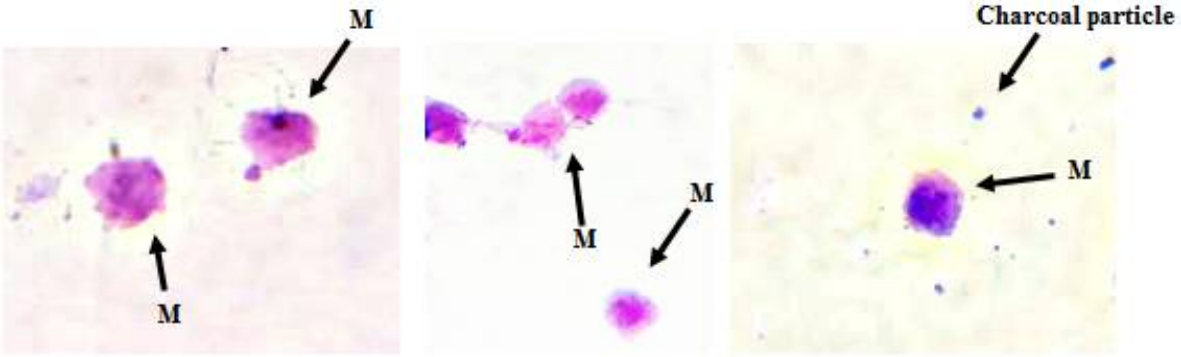

Figure 3: Giemsa stained morphology of inflammatory Macrophages (M) presented in the exudate collected from swim-bladder (x400). Macrophages showed pseudopodia like growth.

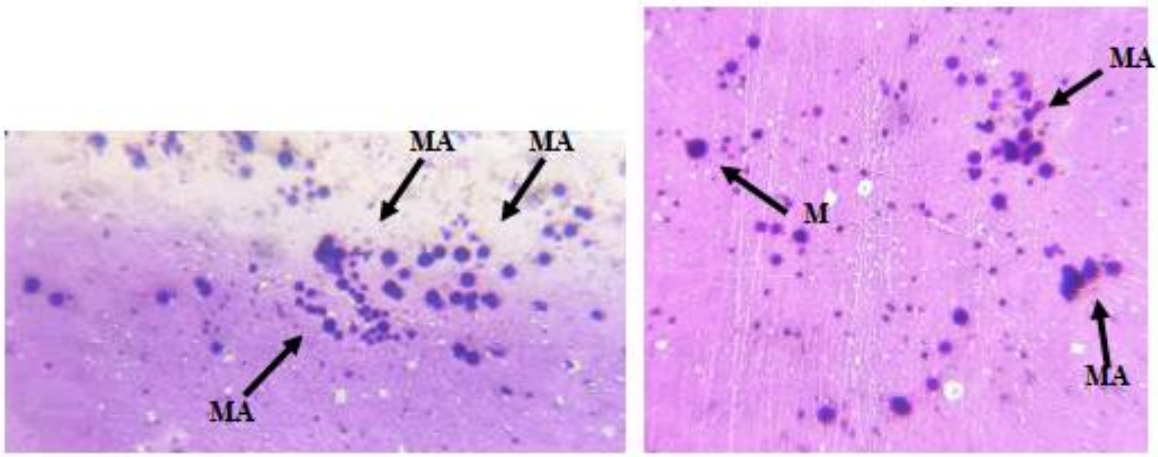

Figure 4: Aggregation of leishman's eosin methylene blue solution stained Macrophages (M) noticed in the exudate collected from swim-bladder (x400), MA=Macrophage Aggregates

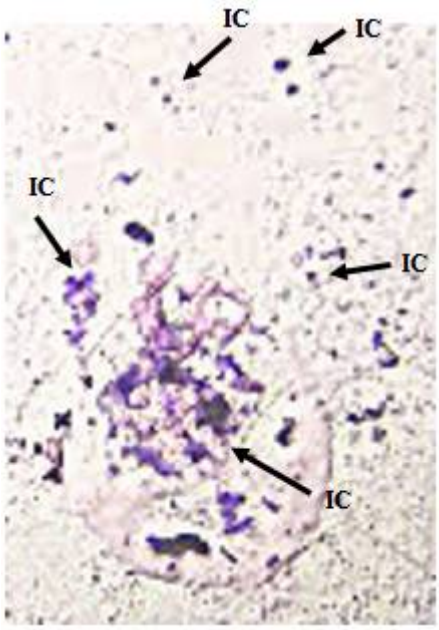

Figure 5: Aggregation of Giemsa stained inflammatory cells (IC) presented in the exudate collected from swim-bladder (x100)

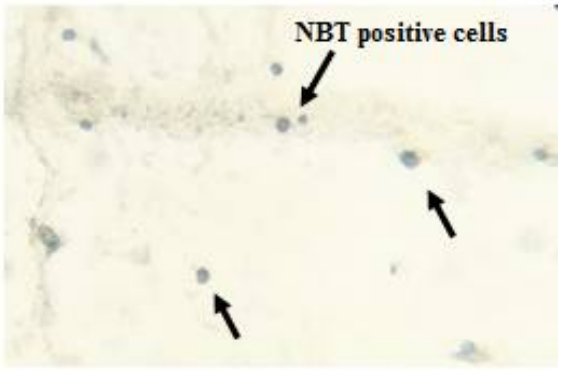

Figure 6: Nitroblue Tetrazolium (NBT) positive cells indicated reactive oxygen species (ROS) in the exudate from swim-bladder (x400)

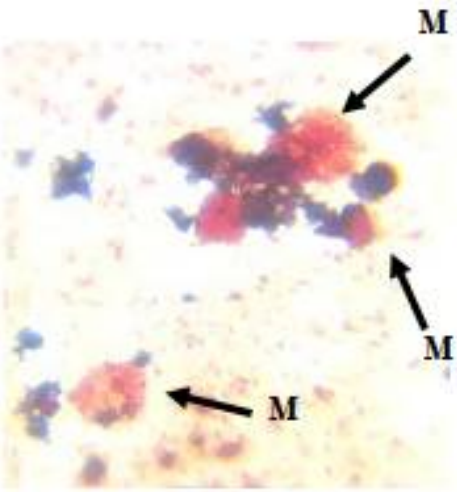

Figure 7: Neutral red stained inflammatory Macrophages (M) presented in the exudate from swim-bladder (x400)

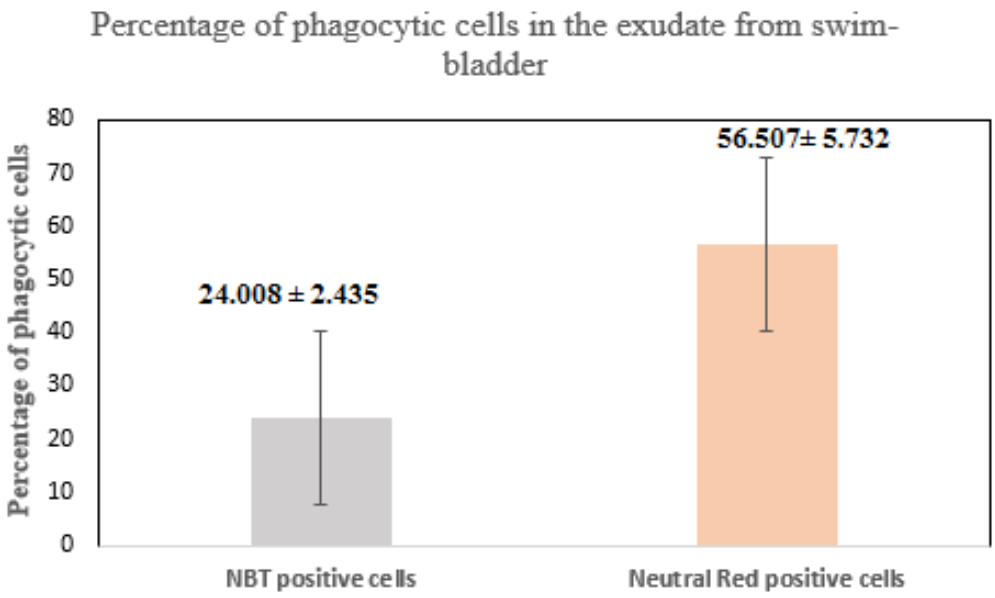

Figure 8: Percentage of phagocytic cells in the exudate from swim-bladder. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

DISCUSSION

Hematological parameters assessments may be important indicators of fish health status [7].

Yu *et al*., (2022) showed that teleost swim-bladder (SB) possesses mucosa-associated lymphoid tissue (MALT) with striking structural and functional immune commonalities with that of lung and other Type-I mucosal surfaces [8]. They found that infection of the SB with virus elicited a strong immune response in the SB, and that reinfection induced local Ig T+ B-cells proliferation in the SB as well as virus-specific s Ig T (immunoglobulin for Teleost) [8]. Teleost fish represent the oldest bony vertebrates featuring MALT and immunoglobulins (Igs) [9]. It was previously shown that teleosts contain IgT, the most ancient Ig specialized in mucosal immunity against pathogens [10]. Moreover, it was demonstrated that, likely to mammalian IgA, teleost secretory IgT (sIgT) is the main sIg isotype coating the microbiota of mucosal surfaces [11].

Matsuyama *et al*., 1999 reported tilapia neutrophils were collected from the swim bladder 24 h after injection with *Escherichia coli*. To collect inflammatory macrophages, the fish were injected with 0.2 ml neutrophil lysate into the swim bladder [6]. In mammals, Ward, (1968) reported that the neutrophil lysate was chemotactically active for macrophages [12].

Carrageenin (marine colloids) injection caused accumulation of inflammatory cells into the swim bladder of Nile tilapia, as reported by Dotta *et al*., 2011 [13]. The circulating blood cells participated in inflammatory response included thrombocytes, lymphocytes, macrophages and granulocytes [13].

In fish, thioglycolate were tested in the swim bladder of *Oreochromis niloticus* **[14]** which induced vascular congestion, accumulation of macrophages and granulocytes.

Aggregation of Macrophages (M) and inflammatory cells (IC) were noticed in our experiments (Fig. 4 and 5). Nitroblue Tetrazolium (NBT) positive cells indicated reactive oxygen species (ROS) found in the exudate (Fig. 6). Neutral red positive inflammatory Macrophages (M) indicated presence of lysosomal enzyme (Fig. 7). These cells were related to phagocytosis. Our result also showed inflammatory Macrophages (M) with pseudopodia like growth (Fig. 3).

Neutral red is specifically retained by acidic vesicles like lysosomes and it is frequently used as an indicator of phagocytic cells. NBT staining is used to determine the production of superoxide anion ($O2^{-}$) in various phagocytic cells. NBT becomes reduced by free oxygen radicals forming a blue-black compound. Percentage of phagocytic cells like NBT positive cells and Neutral red positive cells in the exudate from swim-bladder were 24.008% ± 2.435 and 56.507% ± 5.732, respectively (Fig. 8).

The presence of a great number of phagocytic cells found in this assay suggests that fishes injected with charcoal particles showed increased inflammation. This increase of the inflammatory reaction may be important for the organism's defense against infections. These results corroborate the observations of other researchers.

CONFLICT OF INTERESTS

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

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