Research Article

EFFECTS OF SINGLE AND SHORT-TERM EXPOSURE OF RABBIT FISH VENOM ON HAEMATOLOGICAL RESPONSE IN Mugil cephalus

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Abstract

Background: In this study the effect of different dose dependent of Siganus lineatus fish venom on haematological parameters in Mugil cephalus was investigated. Methods: A total of fifteen fish (25.09 ± 2.34 cm length; 78.43 ± 19.54 g weight) were brought from Annakoil. All animals were transferred in tank and acclimatized for a period of 10 days. After this acclimatization period, all fishes were exposed to different dose of S. linatus venom (C - 0.0001 µl/l; T1 - 0.0001 µl/l; T2 - 0.001; T3 - 0.01 µl/l; T4 - 0.1 µl/l; T5 - 0.25 µl/l; T6 - 0.5 µl/l; T7 - 1 ml/l) for 7 days. Blood samples were collected from before treatment and after exposure of venom. Haematological parameters like, Total RBC, Total WBC and Haemoglobin were analyzed. Results: At the end of the experiment, total RBC was recorded in T4 (23.45 x 10⁶ cu/mm³) as well as WBC cells (3.89 x 10³ cu/mm³). The Hb was recorded in highest value in T3 and T4 (7.9) when compared to control. T6 and T7 revealed suppressed RBC, WBC and Hb level when compared to control. Conclusion: The results of this study showed that the low dose of venom enhanced the haematological response in M. cephalus.

Article History

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1. Introduction

Haematological and biochemical parameters are the two most vital tools used by fish biologists and researchers in many parts of the world. These parameters are closely related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on the blood characteristics (Fernandes and Mazon, 2003). Blood parameters have been used as indices of fish health status in a number of fish species to detect physiological changes as a result of stress condition such as transportation, handling, hypoxia and acclimation (Akinrotimi, 2006; Alwan et al., 2009). Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. In mullet Mugil cephalus Linnaeus, 1758, the haematological and biochemical parameters were previously studied in relation to different habitats (Fazio et al., 2012).

Fishes are one of the diverse sources of natural products and bioactive compounds with over 40,000 known species. They combat...
infections caused by viruses, bacteria, fungi and parasites that are similar to those of humans and other vertebrates. There are more than 200 fish species reported to produce venoms, but less than 20 have been studied in some detail. Most of these venoms appear to comprise proteins and peptides as well as other pharmacologically active substances. The significance of fish venoms both for research and medical therapeutic purposes and as chemical defenses remains poorly appreciated. The number of compounds isolated from various marine organisms has soared from 10,000 in 2001 to 14,000 in 2007. The Rabbit fish *Siganus linateus* is considered to be one of the highly venomous fishes in the world and certainly the most venomous in the *Siganidae* family. So far, literature on exposure of different doses venom on haematological response in Mullet fishes is very limited. Therefore, the present investigation was undertaken to analyze the effect of different dose exposure of venom *S. linateus*, in *M. cephalus*.

2. Materials and Methods

Experimental fish and their maintenance

A total 15 *Mugil cephalus* (21.93 ± 2.03 cm fork length, 80.28 ± 21.32 g weight), were brought from Annakoil, Tamilnadu. All fish were confirmed as healthy on the basis of an external examination for any signs of abnormalities or infestation. All sampled fish were then transferred into laboratory where they were acclimatized in rectangular tank (1000 L) with continuous aeration for a period of 10 days. Half of the water in the experimental tank was exchanged on daily basis. During the experiment the fish were maintained under natural photoperiod and they were fed with control diets (46 % crude protein; 20 % crude fat; 10 % ash; 1.5 % fiber) once daily (10:00) at a ratio of 2 % body weight.

Collection of rabbit fish

Fresh marine rabbit fishes *S. linaetus* were collected from Annakoil, South East Coast of Tamil Nadu, India. Collected samples were stored in sterile bottles and examined in fishes in tissue damage, disease infected (eye, gill and skins).

Preparation of crude venom

A pair of venom sacs from the dorsal spine of *S. linaetus* was dissected out and the venom fluid was collected with a syringe. To the venom fluid, 2 ml of distilled water was added. Although a significant amount of insoluble materials was observed, the homogeneous suspension obtained after brief shaking was used as crude venom. In the fish, dorsal spines were cut off. After removal of the integumentary sheath, they were homogenized in buffered saline (0.15 M NaCl in 0.01 M phosphate buffer, pH 7.5) and centrifuged. The supernatant obtained was used as crude venom.

Experimental setup

After this acclimates period, all fishes were exposed to different dose of *S. linatus* venom (C - 0.0001 µl/l; T1 - 0.001 µl/l; T2 – 0.001; T3 - 0.01 µl/l; T4 - 0.1 µl/l; T5 - 0.25 µl/l; T6 - 0.5 µl/l; T7 - 1 ml/l) for 7 days. Blood samples were collected from before treatment and after exposure of venom. Haematological parameters like, Total RBC, Total WBC and Haemoglobin were analyzed.

Collection of Blood

After 15 days of acclimation, all fish were anaesthetized using MS-222 (Merck, India) at the concentration of 500 mg/L. Blood was immediately collected by puncturing the caudal vein using a 20 G × 1 ½ syringe and collected into different eppendorf tubes, one eppendorf tube (1.5 ml, Torsan) containing EDTA (2.7 %) as an anticoagulant agent for the assessment of haematological parameter.

Haematological parameters

Haemoglobin was determined by Sahli’s method (Dethloff et al., 1999). Red blood cell (RBC) and white blood count (WBC) counts were determined using a haemocytometer with Neubauer counting chamber as described by Blaxhall (Blaxhall and Daisley, 1973). The following formula was used to calculate the number of erythrocytes and leucocytes per milliliter of the blood sample:
Number of cells = Number of cells counted × dilution/Area counted × depth of fluid

3. Results

RBC counts of Groups T3, T4 and T5 were significantly higher than control. In the T6, and T7 group, RBC counts as well as WBC and Hb were decreased. In the 7th day of the experiment, T3 and T4 showed the highest RBC count than other treatments. Exact variations were observed in the treatments, when compared to control group (Figure 1). During the study, the control group of WBC count was normal. In the T3 and T4 group, WBC counts were increased in the 7th day of the experiments and the WBC count was decreased in T6 and T7 group fishes (Figure 2). In the experimental group, the highest Haemoglobin was found in T4. The T7 fish showed a least haemoglobin in the experiments when compared with control and other group (Figure 3).

4. Discussion

The hematological studies in fishes have assumed greater importance because these parameters were used as an efficient and sensitive index to monitor the physiological and pathological changes induced by natural or anthropogenic factors such as bacterial or fungal infection or pollution of water resources (Blaxhall and Daisley, 1973). Blood parameters were considered as a useful tool in diagnosing the functional status of the body in response to various stressors (Chekrabarthy and Benerjee, 1988). The toxicants are stressors which are accumulated in the fish through the food chain or absorption through the general body surface and severely affect the life supporting system at molecular and biochemical levels. The Pollutants generally produce relatively quick changes in hematological characteristics of fish (Johansen et al., 1994; Rizkalla et al., 1999).

The quality of water and the well being of fishes are interconnected and directly proportional. The fluctuations in any of the parameters severely affect the dwelling organisms, especially fish (Greig et al., 2005). Even slight variations in water quality cause a wide variety of stresses among fishes because their homeostatic mechanisms are highly reliant on existing conditions in their immediate surrounding parameters (Nussey et al., 1995). In general, there exists much variation among fishes to adapt to alterations in salinity and is often proportional to the pace of the changes. In natural settings, salinity levels can fluctuate with tides, season, or evaporation from surface waters. Similarly pH also plays a significant role in metabolism, maintenance of homeostasis and physiological well being of aquatic animals (Wood et al., 1989; Parra and Baldisserotto, 2007).

In this context, the marine, toxic and venomous fishes are storehouse of secondary metabolites. In addition to venom apparatus, many species of fish are able to secrete substances from their skin, known as ichthocrinotoxins, which are capable of repelling or incapacitating other marine animals (Klaassen and Watkins, 1999). These secretions are also reported to possess antibiotic activity (Thulesius et al., 2009). Antimicrobial activity of venom of catfish *Cathorops spixii* and many other fish mucous have been reported (Ramos, 2009). Likewise, jelly fish (*Aurelia aurita*) toxin was also assessed for antimicrobial activity (Ovchinnikova et al., 2006) novel pharmacological compounds and therapeutic agents. Studies on antimicrobial bioactivity of fish venoms against human bacterial and fungal pathogens are scanty in India. Hence, this present work has been attempted to different doses of *S. linatus* on haemtaological parameters like Total RBC, WBC and Hb in *M. cephalus*.

The variation degree on the haematological response is an important tool for fish health diagnosis and may vary according to stressor stimulus, treatment, parasitic or infectious diseases (Chen et al., 2004). In the present study, infected fish RBC counts were decreased in the fish. Decreased RBC counts and hematocrit concentration indicate that RBCs are being destroyed by the leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis. A decline in RBC and hematocrit combined with signs of anaemia was also described by Hoffmann and Lommel in cases of
Fig 1: Dose dependent effect of rabbit fish venom on total erythrocyte count in *Mugil cephalus*

Fig 2: Dose dependent effect of rabbit fish venom on total leukocyte count in *Mugil cephalus*

Fig 3: Dose dependent effect of rabbit fish venom on total haemoglobin level in *Mugil cephalus*
proliferative kidney disease (Hoffmann et al., 1984) T3, T4 treated groups, showed an increased blood count when compared with control diet. WBC, RBC, haemoglobin B and thrombocytes were decreased in the infected fish when compared with control group. Waagbo (Waagbo et al., 1988) reported that in Atlantic salmon, Salmo salar with the ‘Hitra disease decreased in the values of RBC, hematocrit and haemoglobin B, associated with symptoms of severe Anaemia. Cardwell and Smith (1971) did find a progressive effect on hematocrit and haemoglobin B in juvenile Chinook Salmon with vibriosis. Harbell et al. (1979) recorded the same in coho salmon, Oncorhynchus kisutch experimentally infected with a highly virulent Vibrio anguillarum. In our present study, high dose of venom suppress the WBC, RBC, hemoglobin level. Harikrishnan et al. (2003) were showed related increased WBC counts and also reported that decreased RBC counts and hematocrit indicated that erythrocytes were being affected or destroyed with the infection.

Addition of unwanted substances into the water bodies cause changes in the physical, chemical and biological characteristics of the aquatic system which lead to ecological imbalance. The industrial effluents contribute a lot to water pollution forming a threat to aquatic plants and animals (Ramona et al., 2001). A greater part of the pollutants exhibit biomagnification and bioaccumulation capabilities with a broad spectrum of impacts, and stresses on aquatic organisms (Censi et al., 2006). The pollution leads to a steady decline in the aquatic flora and fauna, particularly fishes. Wedemeyer (1996) reported that the fishes are more susceptible to stress than many other animals because of their intimate dependence upon their surrounding environment.

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5. References


