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HEMATOLOGICAL AND HISTOLOGICAL INVESTIGATIONS ON HEALTHY AND SAPROLEGNIA SP. INFECTED CLARIAS GARIEPINUS (BURCHELL1822)

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ABSTRACT

Present study deals with the comparative study of hematological parameters of healthy and *Saprolegnia sp*. infected *Clarias gariepinus*. For this study 10 healthy and 15 infected fishes measuring average values of 16 ± 3 cm in length and 13 ± 3 gm in weight were used. Significant changes were observed in Hemoglobin content, Red Blood Cell count, White Blood Cell count, Packed Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin concentration and differential count of WBCs. It was observed during the study in *Saprolegnia* infected fish there was a significant ($P < 0.05$) decrease in hemoglobin (Hb) content, Red Blood Cell count (RBCs), Packed Cell Volume (PCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin concentration (MCHC), lymphocytes and Eosinophils percentage. While Mean Corpuscular Volume (MCV), White Blood Cell count (WBCs) and Neutrophils percentage and Monocytes were found significantly increased as compared to healthy fish. Histological studies of infected tissues showed various types of destructions due to hyphal penetration deep into muscular layer, spores were observed in underlying musculature and granulomas were formed with fibrillar layers. Infection was observed in form of fungoid patches and lesions.

Keywords: *Clarias gariepinus*,. hematological parameters, *Saprolegnia sp*.

INTRODUCTION

Clarias gariepinus is a commercially cultured fish and stressors such as handling, overcrowding and water quality always associated with the culture system. Under stressed conditions aquatic fungi can easily attack fish. Many fungi are pathogenic to fish and most common among them is Saprolegneous fungi. *Saprolegnia* infection in fish has been reported earlier by Roberts et al [1], Chauhan [2] and Chauhan et al [3]. Some other workers like Bruno [4] and Hatai and Hoshiai [5] also reported *Saprolegnia* infection in fish. The infection spreads very quickly and leads to mortality and causes huge loss at every stage of fish especially in brood stocks Stueland et al [6] and Howe and Stelhy [7].

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Hematological parameter provides the physiological state of fish health. Mycotic infection affected the blood parameters of fish have been reported by Banerjee and Bhagat [8], Qureshi et al [9] and Shah [10]. Since fungi are pathogenic to fish, it causes various levels of destruction in tissue. To find out the extent of destruction histopathological studies are important.Some workers reported histological variations in fish tissue were Hatai [11], Refai *et al* [12] and Chauhan et al [3]. The present study was designed to investigate the changes in hematological parameters of *Saprolegnia* infected fish and histological studies to find out the extent of infection.

MATERIALS AND METHODS

For this study a total number of 10 healthy and 15 mycotic infected *Clarias gariepinus* fishes measuring average values of 16 ± 3 cm in length and 13 ± 3 gm in

weight were collected from Sarangpani Lake, Bhopal. Fishes were brought to the laboratory for further examination. Fungal cultures were prepared by taking small innocula from different infected portions of fish body.

Cultures were prepared on Sabourauds Dextrose Agar (SDA) and Potato Dextrose Agar (PDA).Growth was observed by incubating them at temperature 15-18°C. All the cultured isolates were identified as *Saprolegnia sp.* Cultures were identified with the help of keys of Coker [13] and Khulbe [14].

For hematological examination ten healthy and ten infected fishes were used. Blood was drawn from caudal peduncle into Di Potassium EDTA containing tube by the process as described by Hrubc & smith [15]. RBCs and WBCs were counted by haemocytometer and values were calculated as $10^6/mm^3$ and $10^3/mm^3$ [16]. Hemoglobin content was determined by using hemoglobin test kit (DIAGNOVA, Ranbaxy, India). MCV, MCH and MCHC were calculated by following the methods of Dacie and Lewis [17]. The blood film was prepared and stained with Giemsa stain for morphology, micrometry and differential count of leucocytes. All the values of healthy and infected fishes were analyzed by students't' test.

For histological examination, tissues of five infected fishes were examined. Tissues were fixed in aqueous Bouin's fluid for 48-72 hours .The tissues were then processed routinely and prepared into paraffin blocks. The blocks then cut into 4-6µm thickness and stained with haematoxylin and eosin. Slides were observed under microscope to study the changes. Standard histological methods of Roberts [18] were followed for investigations.

RESULTS

A total number of fifteen *Saprolegnia sp.* Infected *Clarias gariepinus* were studied for hematological parameters. Infected fishes showed lost epidermis, white fungoid patches and ulcerations on body. (Fig 1&2).

Various changes were observed in the blood parameters of infected *C. gariepinus* as compared to normal fish. A significant decrease ($P< 0.05$) in the percentage were observed in the values of hemoglobin content (18.8%), Red Blood Corpuscles (20.4%), Packed Cell Volume (9.7%), Mean Corpuscular Hemoglobin (8.23%), Mean Corpuscular Hemoglobin Concentration (7.54%), Eosinophils (17%) and Lymphocytes (20.2%) of infected fish.

A significant increase ($P<0.05$) were observed in the values of White Blood Corpuscles (10.12%), Mean Corpuscular Volume (8.27%) , Neutrophils (10.2%) and Monocytes (5.46%) as compared to normal fish (Table 1).
Histopathological studies of infected tissue of five specimens showed varying degree of destructions. Growth of fungal hyphae was observed on hypodermal layer, encysted zoospores were observed in underlying musculature, muscle cells lost their original appearance and cells accumulated to form granulomas like structures. In severely infected fish fungal hyphae found penetrating deep in muscular layer. (Fig 3 to 6).

Table 1. Hematological parameters of normal and *Saprolegnia sp.* infected *Clarias gariepinus.*

S. No.	Parameters	Units	Control fish	*Saprolegnia* infected fish	% change
1.	Hemoglobin	g/dl	14.26 ± 0.126	12.61 ± 0.132	- 18.8 S
2.	RBC count	$10^3/mm^3$	3.68 ± 0.421	2.93 ± 0.234	- 20.4 S
3.	WBC count	$10^3/mm^3$	11.54 ± 0.16	12.83 ± 0.27	+ 10.12 S
4.	MCV	mm^3	142.09 ± 0.21	154.78 ± 0.14	+ 8.27 S
5.	PCV	%	48.64 ± 0.67	44.35 ± 0.34	- 9.7 S
6.	MCH	Pg	42.11 ± 0.12	39.27 ± 0.65	- 8.23 S
7.	MCHC	g/dl	31.42 ± 0.16	29.21 ± 0.38	- 7.54 S
8.	Neutrophils	%	16.36 ± 1.02	18.21 ± 0.62	+ 10.2 S
9.	Eosinophils	%	77.08 ± 0.69	64.21 ± 1.02	- 17.0 S
10.	Lymphocytes	%	3. 44 ± 0.21	4.31 ± 0.24	- 20.2 S
11.	Monocytes	%	2.12 ± 0.96	2.24 ± 1.02	+ 5.46 S

Data are the average ± standard Error values of ten controls and fifteen infected estimated fishes. S= ($P \leq 0.05$).

Fig 1 & 2. Showing *Saprolegnia sp.* infected *C.gariepinus* with lost epidermis and infected fins. Lesions on body surface with hyphal growth.

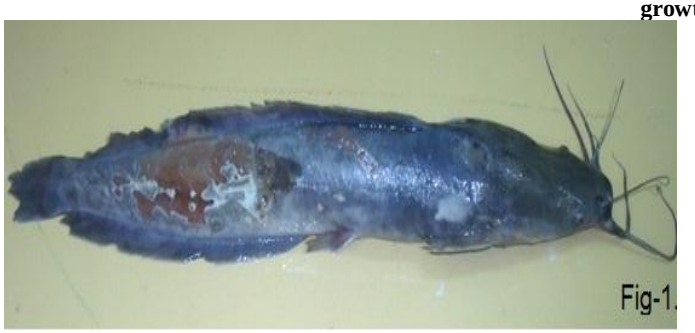
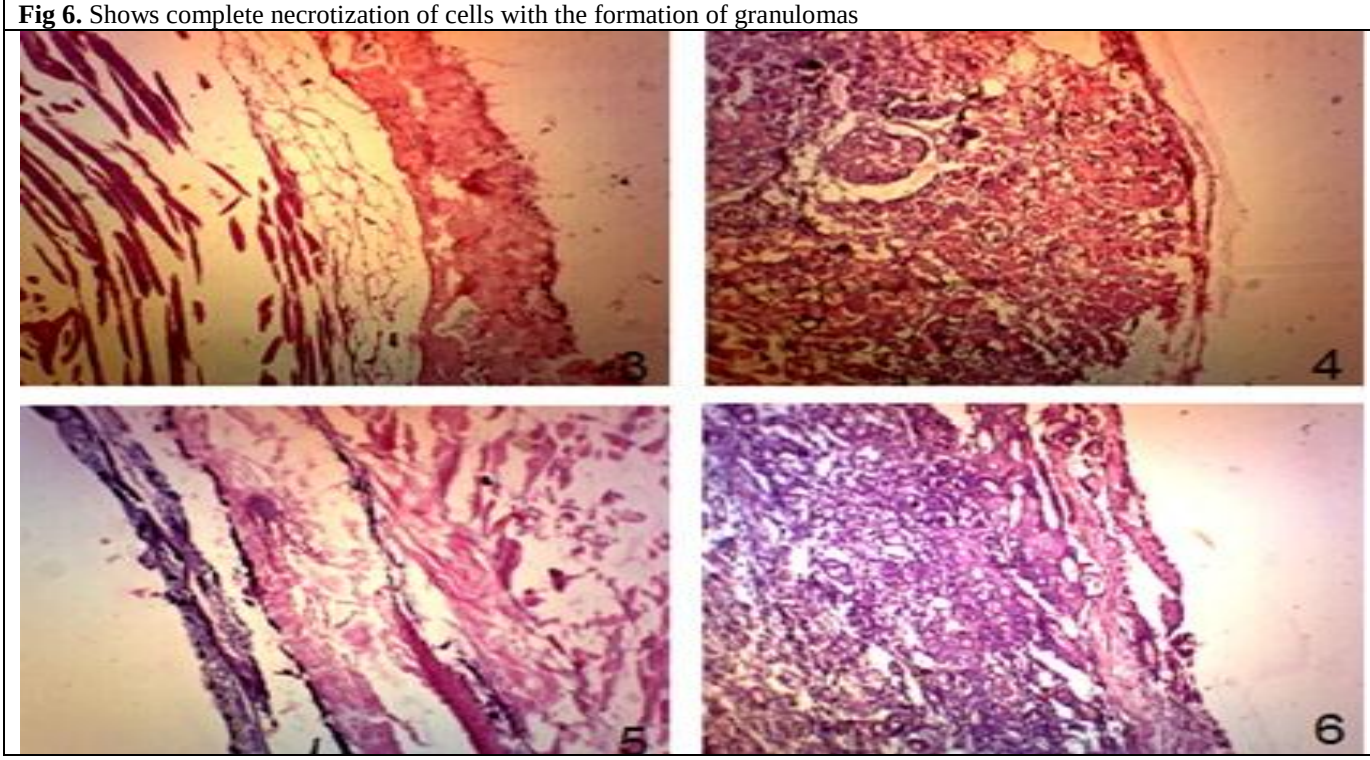


Fig-1.


Fig-2

Fig 3. Shows necrotized hypodermis with hyphal growth and degenerated muscles.
Fig 4. Shows lost epidermis and accumulated muscle cells with spores.
Fig 5. Shows destruction of different layers of skin.
Fig 6. Shows complete necrotization of cells with the formation of granulomas



DISCUSSION

During the present study *Saprolegnia sp*. have been isolated from *C.gariepinus*. *Saprolegnia* have also been isolated from Channel cat fish by Robert et al [1]. It is evident from the observations that *Saprolegnia sp*. infection caused several changes in hematological parameters .Decrease in hemoglobin content and red blood cells showed anemic characters of the fish which leads to hemodilution and immunosuppression due to which infection grows faster and fish leads to mortality . Anemia due to *Saprolegnia* infection in different fish species have been reported by Shah and Altindag [19] and Shah [10].

Decrease in hemoglobin trend may be a result of swelling of RBCs as well as poor mobilization of hemoglobin from spleen. The decrease in WBCs has been reported due to increased secretion of corticosteroids and hemodilution [20]. In present study also WBCs were found to be increased in infected fish. An increase in percentage of Neutrophils and Eosinophils due to infection is reported by Shan *et al*. The increase in number of granulocytes in infected fish may be due to increase in tissue damage by pathogens or other stress factors [21, 22] and Qureshi et al also reported the changes in similar patterns as in the present study. A decrease in lymphocytes and monocytes percentage was observed which is similar to the findings of Alverez et al [23].

Varying degree of histopathological alterations have been observed in the tissues of *Saprolegnia* infected *C gariepinus* like loss of epidermis, necrotized hypodermis with hyphal growth and completely destroyed musculature. Similar types of changes in the tissue of *Saprolegnia* infected fish have been reported by Hatai [13], Hatai et al [24] and Hussian et al [25].

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