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Pathological and Biochemical Studies in gray mullet Infected with *Saprolegnia parasitica* and Methods of Treatment

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ABSTRACT

The present study was planned to investigate the effect of Saprolegnia parasitica infection in the hematological, serum biochemical and pathological alterations of gray mullet. Forty five fish were divided into three equal groups. Fish of first group served as a control. Fish of group (2&3) were infected by Saprolegnia parasitica. Fish of group (3) were treated after 7 days of post-infection using sodium chloride 2ppm and 1% Nigella sativa for 10 days. Sampling was done after 1 and 7 days of post-infection (gps 1 & 2) and 10 days of post-treatment (gps 1& 3). The results revealed a non significant changes in the hematological and the biochemical parameters after 1 day of infection, but after 7 days of post-infection and 10 days of post-treatment, a significant decrease in RBCs, Hb, PCV and significant increase in AST, ALT, urea, creatinine, sodium, potassium, cortisol, insulin and glucose were seen. Iron showed a significant decrease at the same period of sampling. The pathological examination revealed a massive fungal growth resembling a tuft of cotton wool threads was seen in eyes, grills, fins and in localized areas of the skin. Microscopically, the fungal hyphae and spores appeared on eyes, gills, skin and underlying muscles with marked degenerative, necrotic and inflammatory reactions. These reactions were evident, after 7 days of post-infection and the severity of the lesions were markedly decreased after 10 days of post-treatment. It could be concluded that, saprolegnia parasitica infections induced marked tissue alterations as well as some hematological and serum biochemical changes. Although sodium chloride treated the infected cases and allowed the regenerative processes but it does not progress the hematological and serum biochemical parameters.

Key words: gray mullet; *Saprolegina parasitica;* Biochemical changes, sodium Chloride, *Nigella sativa*

INTRODUCTION

Saprolegnia species are opportunistic facultative parasite either ecrophs or saprotrophs [1]. It causes substantial mortality among fresh water fish and mostly associated with environmental stresses such as overcrowding. Rough handling, transport, low dissolved oxygen, temperature fluctuation, osmotic shock and water pollution [2]. Moreover, saprolegnia may be secondary invader to bacterial infection or parasitic agents [3]. However, the importance of saprolegnia as a primary pathogen is still debatable where some outbreaks with mass mortalities may occur absence of other pathogens [4].

Saprolegniosis in fish usually starts as a cotton wool like, white to dark gray or brownish growth over the head region or dorsal fin and then spread allover the body. The infection may be associated with pathological and hematological alterations as well as biochemical changes [5]. Sodium chloride is used in protection of fish from

ectoparasites and it is reported to be a strong antifungal [6]. The present work aimed to study the effect of *saprolegnia parasitica* on biochemical and clinicopathological findings of infected gray mullet before and after treatment with sodium chloride.

MATERIALS AND METHODS

Fish: Forty five gray mullet with average body weight of 100-150 gm/fish were obtained from Fish Farm and transported to the laboratory and reared in 3 equal glass aquaria (115 liter capacity), fed a balanced ration and provided with continuous aireated and renued tap water.

Fish were kept one week for acclimatization and mean time subjected to mycological, bacteriological and pathological examinations.

Fungus: *Saprolegnia parasitica* was kindly obtained from Mercen Department, Faculty of Agriculture, Ain Shams University.

Chemical: Sodium chloride and *Nigella sativa* oil was obtained from Nasr. Co., Cairo, Egypt.

Dose: Sodium Chloride 2ppm and *Nigella sativa* add as 1% in commercial ratio

Experimental infection: Fish were divided into 3 equal groups. Fish of group (1) were kept without treatment to serve as a control. Fish of groups (2 and 3) were infected by *Saprolegnia parasitica.* Fish of group (3) were treated for 10 days. The challenge infection was done by immersing a manual wounded gray mullet in a zoospore suspension of *Saprolegnia parasitica* (4×10^6 zoop/L) for 10 min. according to Willoughby and Pichering [7]. Infection was indicated by the presence of cottony white patches on the body of fish and diagnosed using G.Y.Ps. agar plates.

Sampling: Blood samples and tissue specimens were taken at first and seventh days of infection (gp2) and also after ten days of treatment (gp3). Sampling was also done at the same tome from control group (gp1). Blood samples were taken in heparinised microhematocrit tube and other tubes to be centrifuged at 3000 r. p. m. for 10 min. for serum separation. The serum stored at 20^oC until analysis.

Hematological examinations: The erythrocytic indices (RBCs, Hb, PCV and MCV) and reticulocytes were determined according to Schalm [8].

Serum biochemical analysis: Serum aspirate aminotransferase (AST) and alanin aminotransferase (ALT) also serum urea, creatinine and glucose were estimated using kits supplied from Biomerieux (France). Sodium and potassium were determined by flame photometer according the method described by silversmith [9]. Serum cortisol level was determined using radio immunoassay technique [10]. Insulin was estimated by radioimmunoassay using kits obtained from diagnostic products corporation (Los Angeles, USA). Iron was determined using atomic absorption according to Joseph and Roger [11].

RESULTS

Saprolegniosis, after 1 of day post-infection, induced non significant changes in the hematological and serum biochemical parameters. A significant decrease in RBCs, Hb and PCV was observed in gray mullet, after 7 days of post-infection and 10 days of post-treatment, while MCV and a period of reticulocytes showed a high significant decrease at the same period of sampling in comparison with control. A significant increase in AST, ALT, urea, creatinine, sodium, potassium, cortisol, insulin and glucose was noticed in gray mullet after 7 days of post-infection by *Saprolegnia* and 10 days of post-treatment while iron showed a significant decrease at the same period of sampling in comparison with control.

Clinically, gray mullet infected by saprolegnia showed conspicuous fungal colonies, after 7 days of post-infection that appeared on the mouth, gills, eyes, fins and localized areas of the body surface. The fungal growth appeared white or grey thin threads resembling a tuft of cotton wool. The colour frequently changed to dark by accumulation

of debris. Blindness was evident, in some cases, due to eye infection. Later on, some gray mullet swam eradically and vigorously into the side of the aquaria. After 10 days of treatment most of these signs were disappeared.

Grossly, massive fungal growth appeared on the fins, gills and skin. It is associated with focal areas of hemorrhage, necrosis and ulceration. The internal organs revealed a mild congestion. Small grayish white foci on the liver surface was seen.

Table 1: Effect of Saprolegniosis on some hematological parameters of gray mullet before and after treatment in comparison with control (Mean±SE)

Parameters	Control gp.	Infected gp.		Treated gp.
		1 day P.I.	7 days P.I.	(10 days P.T)
RBC_s ($10^6/mm^3$)	2.22±0.10	2.33±0.07	1.45±0.85*	1.33±0.24*
Hb (gm/dl)	8.03±0.24	8.21±0.04	7.30±0.63*	6.93±0.52*
PCV	18.53±0.74	17.51±0.05	15.51±0.33*	14.51±0.18*
MCV (FL)	34.03±0.04	33.28±0.06	28.10±0.52**	27.01±0.44**
Reticulocytes (%)	1.55±0.04	1.44±0.08	1.13±0.13**	1.45±0.18**

***Significant at P<0.01, P.I. = Post-infection, P.T. = Post-treatment, gp. = group*

Table 2: Effect of Saprolegniosis on some serum biochemical parameters of gray mullet before and after treatment in comparison with control (Mean±SE)

Parameters	Control gp.	Infected gp.		Treated gp.
		1 day P.I.	7 days P.I.	(10 days P.T)
AST (U/L)	79.00±0.64	79.00±0.17	124.00±0.40	126.00±0.63*
ALT (U/L)	20.00±0.19	21.00±0.17	31.00±0.18*	36.00±0.14*
Urea (mg%)	3.03±0.34	3.07±0.36	4.05±0.62*	4.63±0.84*
Creatinine (mg%)	0.68±0.21	0.70±0.33	0.90±0.22*	0.96±0.13*
Sodium (mfg/dl)	126.00±0.33	136.00±0.80	148.00±1.12*	162.90±1.82*
Potassium (mfg/dl)	3.80±0.22	3.90±0.40	5.92±0.82*	6.80±0.72*
Cortisol (µg/dl)	0.60±0.18	0.65±0.19	1.42±0.68*	1.71±0.70*
Insulin (µg/dl)	9.20±0.14	10.60±0.70	12.20±0.42*	12.90±0.63*
Glucose (mfg/dl)	59.30±0.34	60.80±0.70	76.00±0.73*	78.80±0.70*
Iron (mg/dl)	215.00±0.17	219.00±1.12	207.00±1.13*	193.00±1.15*

**Significant of p<0.01*

DISCUSSION

It is apparent that, gray mullet infected with saprolegnia caused a significant increase in glucose and insulin levels only during 7 days of infection and 10 days of treatment with potassium permanganate (2.5 mg/L). It is well known that, any stress factor such as handling, incubation, or anathesia have been shown to cause hyperglycemia followed by hyperinsulinemia [7].

The present work revealed that, serum glucose was elevated during 7 days of infection and 10 days of treatment. One consistent effect of cortisol was the reduction in the haemoglobin, PCV, RBCs and iron level as a result of decrease in appetite in the gray mullet or more likely to be the direct of catabolic effect of cortisol on the fish [12].

The experiment showed that sodium (Na) and potassium (K) concentrations were significantly increased, this retention may be attributable to kidney impairment where the kidney is the normal pass way for Na and K, this may explain the main cause for elevation of serum creatinine and urea in the treated groups which also microscopically exhibited vacuolar degeneration of renal tubules. This confirms the previous results recorded by Osfor *et al*. [13], Zaki *et al*. [14] and Abdel Aziz *et al*., [15]. This led to temporal changes in plasma insulin concentration which did not mirror those for glucose. One of the reasons may be the high sensitivity to glucose of pancreatic cells producing somato statin which in turn inhibits insulin secretion during the initial period after saprolegnia challenge [16]. Saprolegnia infection causes a significant increase of cortisol level which may be due to the activation of hypothalamus pituitary internal axis. These results coincide with those observed by Jauncey and Ross [17] and Zaki et al.,[18], who stated that, hyphae of saprolegnia may invade deep tissues of the fish and penetrate the vital organs as kidney, liver and even the central nervous system and eye.

Marked elevations were noticed in the activity of (AST) and (ALT). The liver is the primary organ of detoxification as well as a major site for detoxification reaction, therefore, a significant increase in liver enzymes suggests

explanations for the presence of the saprolegnia parasitica or its toxins in liver. This picture was confirmed histopathologically by the marked vacuolar degeneration of hepatocytes.

As primary pathogen for stressed fish, this is in agree with Zaki *et al.,* [14] and Badran *et al*., [19]. Who stated that hyphae of saprolegnia may invade deep tissues of fish and penetrate the vital organs even the central nervous system.

The clinical signs and postmortem lesions that reported among infected gray mullet were similar to those reported by Aly and Ashram [3] and Attia [20] and Ferguson [21].

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