

Pathological and Biochemical Studies in *Tilapia nilotica* Infected with *Saprolegnia parasitica* and Treated with Potassium Permanganate

¹Mona S. Zaki, ²Olfat M. Fawzi and ³Jaken El-Jackey

¹Department of Aquaculture, National Research Center, Dokki, Giza, Egypt

²Department. of Biochemistry, National Research Center Dokki, Giza, Egypt

³Department of Microbiology, Veterinary of Medicine, Cairo University, Egypt

Abstract: The present study was planned to investigate the effect of *Saprolegnia parasitica* infection in the hematological, serum biochemical and pathological alterations of *Tilapia nilotica*. Forty five fish were divided into three equal groups. Fish of first group served as a control. Fish of groups (2&3) were infected by *Saprolegnia parasitica*. Fish of group (3) were treated after 7 days of post-infection using potassium permanganate 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 1day 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, gills, 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 potassium permanganate treated the infected cases and allowed the regenerative processes but it does not progress the hematological and serum biochemical parameters.

Key words: *Tilapia nilotica* % *Saprolegnia parasitica* % Biochemical changes

INTRODUCTION

Saprolegnia species are opportunistic facultative parasite either ecrophs or saprotrophs [1]. It causes substantial mortality among fresh water fish and mostly associated with enviromental stresses such as overcrowding, rough handling, transport, low dissolved oxygen, temperature fluctuation, osmotic shock and water pollution [2]. Moreover, saprolegnia may be secondary invador 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 the 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 alllover the body.

The infection may be associated with pathological and hematological alterations as well as biochemical changes [5]. Potassium permanganate 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 *Tilapia nilotica* before and after treatment with potassium permanganate.

MATERIALS AND METHODS

Fish: Forty five *Tilapia nilotica* with average body weight of 100-150 gm/fish were obtained from River Nile and transported to the laboratory and reared in 3 equal glass aquaria (115 liter capacity), fed a balanced ration and provided with continuous aired and renewed 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: Potassium permanganate was obtained from Nasr. Co., Cairo, Egypt.

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 after 7 days of infection using potassium permanganate (2.5 mg/L) for 10 days. The challenge infection was done by immersing a manual wounded *Tilapia nilotica* in a zoospore suspension of *Saprolegnia parasitica* (4×10^6 zoop/L) for 10 min. according to Willoughby and Pickering [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 (gp 2) and also after ten days of treatment (gp 3). Sampling was also done at the same time from control group (gp 1). 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°C until analysis.

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

Serum biochemical analysis: Serum aspartate 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 silversmit [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 a non significant changes in the hematological and serum biochemical parameters. A significant decrease in RBCs, Hb and PCV was observed in *Tilapia nilotica*, 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 *Tilapia nilotica* 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, *Tilapia nilotica* 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 *Tilapia nilotica* 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 greyish white foci on the liver surface was seen.

DISCUSSION

It is apparent that, Nile *Tilapia nilotica* 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 *Tilapia nilotica* or more likely to be the direct of catabolic effect of cortisol on the fish tissues [12].

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

Parameters	Control gp.	Infected gp.		Treated gp. (10 days P.T)
		1 day P.I.	7 days P.I.	
RBC _s (10 ⁶ /mm ³)	2.73±0.10	2.83±0.10	1.95±0.24*	1.83±0.14*
Hb(gm/dl)	9.01±0.24	9.21±0.36	8.30±0.73*	7.93±0.62*
PCV	20.01±0.74	19.01±0.17	17.01±0.23*	16.01±0.28*
MCV (FL)	36.27±0.04	35.28±0.08	30.10±0.62**	29.01±0.54**
Reticulocytes (%)	1.75±0.07	1.64±0.06	1.33±0.03**	1.65±0.08**

**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 *Tilapia nilotica* before and after treatment in comparison with control (Mean±SE)

Parameters	Control gp.	Infected gp.		Treated gp. (10 days P.T.)
		1 day P.I.	7 days P.I.	
AST (U/L)	81.00±0.54	81.00±0.63	126.00±0.50	128.00±0.73*
ALT (U/L)	22.00±0.09	23.00±0.07	33.00±0.08*	38.00±0.04*
Urea (mg%)	3.23±0.24	3.27±0.26	4.25±0.72*	4.93±0.94*
Creatinine (mg%)	0.71±0.01	0.73±0.03	0.93±0.12*	0.99±0.23*
Sodium (mfg/dl)	129.00±0.23	139.00±0.70	151.00±1.92*	165.90±1.72*
Potassium(mfg/dl)	4.00±0.23	4.20±0.50	6.62±0.92*	7.30±0.82*
Cortisol (µg/dl)	0.80±0.08	0.85±0.09	1.62±0.78*	1.91±0.80*
Insulin (µg/dl)	10.20±0.74	11.60±0.80	13.20±0.52*	13.90±0.83*
Glucose (mfg/dl)	61.30±0.54	62.80±0.72	78.00±0.83*	80.80±0.90*
Iron (mg/dl)	220.00±0.07	222.00±1.02	210.00±1.23*	196.00±1.05*

CSignificant of p < 0.01

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 passway 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 somatostatin 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]. 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 elevation 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.*, [18]. 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 *Tilapia nilotica* were similar to those reported by Aly [3] and Ashram [19] and Attia and Ferguson [20].

ACKNOWLEDGEMENTS

The authors thank the staff of Mercen in Faculty of Agriculture , Ain Shams University for providing us the strain of *Saprolegnia parasitica*. Mercen is established by Prof. Dr. Saad Ali Zaki , The Ancient Dean of Faculty of Agriculture.

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