Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
2(4):1319-1325
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCHARTICLE

Biochemical and histopathological changes in head kidney of *Labeorohita* infected with *Aeromonasliquefaciens*

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ABSTRACT

Aeromonasliquefaciens is one of the most important pathogen of warm water and freshwater fish affecting the fish culture globally. This organism causes mass mortality in several groups of fish including carps, murrels and catfishes. It is also the etiological agent of several fish diseases including dropsy, hemorrhagic septicaemia, asymptomatic septicaemia, ulcerative infections, tail rot and fin rot. The present study was conducted to estimate the level of protein and DNA and histopathological observations from the head kidney of four groups (66 in each group) of *Labeorohita*. Fish were experimentally infected (intramuscularly) with various doses of *A. liquefaciens* @ 10^{-2} CFU/ Fish (Group A), 10^{-4} CFU / Fish (Group B), 10^{-5} CFU/ Fish (Group C), and 10^{-6} CFU / Fish (Group D). Another four groups (a, b, c and d; 66 in each group) of fish were kept as uninfected controls. Six fish from each group were necropsied, head kidney tissues were separated and analyzed for protein and DNA content at hour 1, 3, 6, 12, 18, 24, 36, 48, 72, 96 and 216 and histopathological changes were made on hour 216. The virulent strain altered the physiology of fish from hour 1 to 216 of experimental period in tissues of head kidney. Head kidney showed decreased values of protein in all the experimental groups from hour 1 to 216 except on hour 1 in group C and on hour 1, 3, 6, 12 and 18 in group D in comparison with controls. Depressed DNA levels were found in all the experimental groups (A, B and C) from hour 1 to 216 except in group B on hour 1 and in group C on hour 1 and 18. Degeneration and necrosis was observed in the head kidney of diseased fish.

Keywords:*Aeromonasliquefaciens, Labeorohita,* histopathological, freshwater fish.

INTRODUCTION

Fish show both non-specific and specific immune response to resist bacterial diseases. Bacterial pathogens infect the host and multiply in the tissues to overcome or avoid these defences. In many diseases in order to resist the infectious pathogens, the immune response of the fish reacts in a multi factorial manner. Although protection is offered by vaccination, there is still much to learn about defence mechanism to achieve protection against many bacterial pathogens. Stimulation of some immune response may be counterproductive by enhancing the adverse mechanisms of thevirulent pathogens in fish (Ellis, 1999). *Aeromonashydrophila* was recognized as a causative agent of ulcer type disease occurred in farmed fishes (Chowdhury, 1998) and frequently isolated from various lesions of epizootic ulcerative syndrome (EUS) of different fishes (Dooly et aI, 1986; Torres et al., 1990; Roberts et al., 1990). Iqbal et al (1998) detected *A.*

hydrophila from EUS affected mrigal. Rashid et al (2008) identified *A. hydrophila* from EUS affected shining Heteropneeustesfossilis. Hasan et al (2008) found histopathological changes in liver and kidney caused by *A. hydrophila* in the fish. Experimental pathogenesis of *A. hydrophila*and histopathological changes were found in experimentally infected shining fish (Mostofa et al., 2008; Islam et al. 2008). Sabur (2006) reported five species of *Aeromonas* bacteria in *Cirrhinus*, *Catlacatla*and*Hypophthalmichthysmolitrix*.
Sarkar and Rashid (2012) reported lowest bacterial counts in the kidney of carps. Satyalatha and VivekaVardhani (2014) found significant alteration in protein and DNA content and histology in the muscle of *Labeorohita* during *A.liquefaciens* infection. The present investigations were undertaken to estimate the protein and DNA content and to understand the histopathology of head kidney in *Labeorohita* infected with various doses of *A. liquefaciens.*

MATERIAL AND METHODS

Labeorohita fish were brought from Nandivelu (Guntur District), AP, India. The fish were divided into 8 groups (experimental and control). All the groups of fish were acclimatized in the laboratory conditions and fed with commercial feed. Pure cultures of bacteria were obtained by streak plate method. Virulent strain of *A. liquefaciens* was procured from MTCC (No. 2654), Chandigarh. 264 fish were divided into four equal groups and were given infection intramuscularly below the region of dorsal fin @10"^{2} CFU / fish (Group A), 10"^{4} CFU / Fish (Group B), *10"^{5}* CFU / fish (Group C) and 10"^{6} CFU/fish (Group D). Another batch of 264 fish (66 in each group) (a, b, c and d) was kept as uninfected control. Six fish from both the experimental and uninfected group were taken and necropsied, total protein and DNA content from head kidney was estimated following by Lowry et al., (195i) and Diphenylamine method. Little bit of tissue was taken for histopathological observations by preserving the tissue in Bouins fluid, sectioned (5mu) and stained by H & E method. Protein and DNA results were subjected to student's' test to find out the significance.

RESULTS AND DISCUSSION

Protein activity:
Fish treated @ 10^{-2} CFU/fish (group A), showed a decrease of protein from hour 1 to 216 when compared to controls. The protein content decreased gradually from hour (62.41 mg/ml) to 18 (57.58 mg/ml) and a slight increase from hour 18 – 216. However, this increase is below normal level. In group B, the level of total protein decreased to below normal level on hour 1 (60.34 mg/ml), 3 (61.72 mg/ml) and 6 (62.41 mg/ml) when compared with the controls (63.80

Table 1 : Content of protein (mg/ml) and DNA (mg/ml) in the head kidney of experimental fish treated with 10^{-2} CFU/ fish (group A), and 10^{-4} CFU /fish (group B) *Aeromonasliquifaciens,* at different periods of infection and controls (group a and group b). Values are expressed in mean derived from five observations.

Hours of necropsy	Experimental groups				Control groups			
	A		B		a		b	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
1	62.41	10.0	60.34	13.33	63.80	11.11	63.80	11.11
3	61.72	9.0	61.72	10.06	63.80	11.12	63.80	11.12
6	58.27	7.77	62.41	8.88	63.78	11.11	63.79	11.11
12	57.93	8.88	63.10	7.77	63.79	11.11	63.78	11.09
18	57.58	10.0	63.79	7.78	63.81	11.09	63.77	11.08
24	60.34	6.66	62.41	7.77	63.80	11.11	63.76	10.99
36	61.03	8.88	61.72	7.76	63.79	11.08	63.79	11.07
48	61.72	11.11	62.06	7.77	63.78	11.07	63.80	11.09
72	62.41	10.0	62.41	6.66	63.77	11.11	63.81	11.11
96	60.34	6.66	62.06	5.55	63.77	11.09	63.82	11.09
216	59.65	4.44	63.10	2.22	63.79	11.11	63.79	11.11

Table 2 : Content of protein (mg/ml) and DNA (mg/ml) in the head kidney of experimental fish treated with 10^{-5} CFU/ fish (group C), and 10^{-6} CFU /fish (group D)*Aeromonasliquifaciens*, at different periods of infection and controls (groups a and group b). Values are expressed in mean derived from five observations.

Hours of necropsy	Experimental groups				Control groups			
	C		D		c		d	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
1	64.13	13.33	65.86	18.88	63.71	11.11	63.79	11.12
3	61.72	10.0	65.17	16.66	63.71	11.14	63.79	11.11
6	61.72	11.11	66.79	15.55	63.70	11.12	63.78	11.13
12	60.34	8.88	65.51	14.44	63.71	11.13	63.79	11.09
18	60.96	14.44	64.13	12.22	63.70	11.14	63.77	11.11
24	61.37	10.0	63.79	16.66	63.71	11.11	63.76	11.12
36	61.06	10.0	62.75	16.66	63.69	11.11	63.78	11.11
48	61.72	7.77	62.06	15.55	63.70	11.12	63.77	11.12
72	60.72	5.55	63.79	14.44	63.71	11.14	63.78	11.13
96	60.68	4.44	62.41	10.06	63.72	11.11	63.79	11.14
216	60.34	4.44	63.79	9.99	63.71	11.11	63.77	11.11

mg/ml). Fish of group C had a protein level which is slightly higher than control value on hour 1 of infection. There is a slight decrease of protein from hour 1 to 216; these values are below normal levels. In experimental fish of group D, the head kidney showed higher level of protein on hour 1, 3, 6, 12 and 18 and this rise reached its zenith on hour 6 (66.71 mg/ml). From hour 24 (63.79 mg/ml) to 216 (63.79 mg/ml) of infection, there was a decrease of DNA content and this decrease is below normal level on hour 48 (62.06 mg/ml) and 96 (62.41 mg/ml).

DNA activity:

In group A, there was a decrease in DNA content from hour 1 to 216, and these values are found to be lower than that of values of uninfected fish (group a) (except the normal level on hour 48). Higher level of DNA was found in group B on hour 1 of infection (13.33 mg/ml) when compared to that of uninfected controls (11.11 mg/ml). From the hour 1 to 216 of infection, there is a decrease in DNA content (10.06 mg/ml) (below normal value). Group C showed mg/ml) and from hour 1 to 12 of experimental period, there was a gradual decline of DNA. From the hour 12 (8.88 mg/ml) to 18 (14.44 mg/ml), a sudden increase of DNA occurred; this value is noticed as peak level of immune response. From hour 18 (14.44 mg/ml) to 36, there is a brisk decrease of DNA (10.10 mg/ml. and decreased gradually from hour 48 to 216). In group D, there was a higher level of DNA on hour 1 and decreased on hour 3 (16.66 mg/ml), 6 (15.55 mg/ml), 12 (14.44 mg/ml), 18 (12.22 mg/ml), 24 and 36 (16.66 mg/ml), 48 (15.55 mg/ml) and 72 (14.44 mg/ml) – but this decrease is higher than that of normal value. From hour 96 (10.06 mg/ml) to 216 (9.91 mg/ml), there is a brisk decrease of DNA (below normal level). Significant decrease of protein in head kidney was found in groups A and C when compared with controls (Table 3). Differences in protein were statistically non-significant in groups C and D when compared with controls and inbetween groups A and B, A and C and B and C. Groups A, B and C showed significant difference when

compared with group D. In comparison with controls there was a significant increase of DNA in head kidney in group D and significant decrease in groups A and B; but there was no significant difference when comparison was made between groups C and controls, and A and C. There had been a significant difference in DNA of group B when compared with group C and in group D when compared with groups A ($P<0.05$), B ($P<0.05$) and C ($P<0.05$).

Histopathological Changes:

The multiple sections of normal heady kidney showed normal parenchyma and interstitial tissue without any proliferation. The renal corpuscle is composed of glomerulus and its capsule showed intact texture. In groups A and B, at day 9 post infection, the head kidney showed congestion of blood capillaries and glomerular tufts with heavy necrotic tissue. The hematopoietic tissue in the kidney was disrupted

Table 2: 't' values obtained for different groups of fish infected with 10^{-2} (group A), 10^{-4} (group B), 10^{-5} (group C) and 10^{-6} (group D) CFU/fish

Experimental (A, B, C and D) and Control (a, b, c and d) groups							
A	aB	bC	cD	d			
Head Kidney Protein							
Mean	60.30	63.76	62.28	63.78	61.34	63.78	64.19	63.78
	A	a	B	b	C	c	D	d
t value	t=6.55*	t=2.29@	t=7.65*	t=0.88@				
	(P<0.05)	(P>0.05)	(P<0.05)	(P>0.05)				
	A	B	A	C	A	D		
	t=2.29@	t=1.67@	t=5.56*					
	(P>0.05)	(P>0.05)	(P<0.05)					
	B	C	B	D	C	D		
	t=2.24@	t=3.60*	t=5.13*					
	(P>0.05)	(P<0.05)	(P<0.05)					
Head Kidney DNA								
Mean	8.49	11.1	7.78	11.01	9.08	11.01	14.64	11.1
A	aB	b	C	cD	d			
t value	t=4.45*	t=4.04*	t=2.01@	t=4.15*				
(P<0.05)	*(P<0.05)*	*(P>0.05)*	*(P<0.05)*					
A	B	A	C	A	D			
	t=2.32*	t=0.51@	t=5.94*					
	(P<0.05)	(P>0.05)	(P<0.05)					
	B	C	B	D	C	D		
	t=2.41*	t=5.79*	t=4.22*					
		(P<0.05)	(P<0.05)	(P<0.05)				

*P value at 5% level of significance is 2.306; *Statistically significant values ; @Statistically non-significant values*

and the glomeruli were enlarged. Heavy infiltration of lymphocytes and macrophages were observed. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed (Fig. 1A). The multiple sections of the head kidney study in group B revealed heavy necrotic tissue. The hematopoietic tissue in the kidney was disrupted by the heavy infestation of bacteria. Glomeruli were enlarged and showed cell proliferation. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed. The glomeruli showed nodular out growths due to severe bacterial infestation (Fig. 1B). In group C, head kidney showed severe necrosis, congestion of the renal capillaries and severe accumulation of granular eosinophilic cells. Accumulation of pus, severe inflammation of sinusoids and serous membrane were observed. Hematopoietic tissue was severely disrupted. Glomeruli were enlarged (Fig. 1C). The multiple sections of the study revealed heavy necrosis and severe blood clots, heavy inflammation and enlargement of glomeruli in fish of group D. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed. Severe infiltration of lymphocytes and neutrophils was observed (Fig: 1D).

The virulent infectious *A.liquefaciens*might have caused abnormality in the level of protein and DNA in head kidney at different hours of experimental period in the present study. Das and Mukherjee (2003) and Begum (2004) also

Figure-1.Histological sections of Head kidney from Control (a) and infected (1A, 1B, 1C and 1D) fish.

suggested that the infectious, pathogenic bacteria may produce ill effects there by disturbing the physiological mechanism of fish. Miyamoto (1976) and Murthy and Devi (1982) also reported that fish exposed to toxicants show impaired protein metabolism. The reduced level of protein at some hours of infection in the infected fish confirm that of Das and Bhattacharya (2006) who reported that the lowered level of protein due to reduction in the synthesis of proteins.

Fish suffering due to aeromoniasis (Groups A, B, C and D) showed atrophy and necrosis in renal haemopiotic tissues as explored by Chein and Chein (1994) in Eel *(Anguilla japonica)* infected by aeromonads species. Mohanty et al., (2008) also explained that head kidney is one of the target organ influenced by *A. hydrophila* and both acute and severe infection cause damage to the head kidney. The changes like necrotic lesion and aggregation of melanin containing macrophages in the head kidney of infected fish (during the entire experimental period) indicate the pathogenic effect of microbial organism in the head kidney; this is an indication of stressful condition of infected fish. These results compare well with that of Mohanty et al., (2008) who also reported necrotic lesions and aggregation of melanin filled macrophages in kidney and spleen of fingerlings of *L. rohita* infected with *A. hydrophilla.*

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DOI: https://dx.doi.org/10.5281/zenodo.7246822
Received: 11 October 2014;
Accepted; 27 November 2014;
Available online : 14 December 2014

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