Biolife 7(2):9-12
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE ORIGINALARTICLE

Aeromoniasis Induced Biochemical and Histopathological Alterations in the Head Kidney of *Labeorohita*

Satyalatha B.D.J.[1*] and VivekaVardhani.V[2]

[1,2]*Department of Zoology Acharya Nagarjuna University Nagarjunanagar-522510*
**Department of Zoology, Government Women's College, Guntur*

*Email:*vadlamudi_vv@yahoo.co.in

ABSTRACT

This study evaluated the effect of multiple doses of *Aeromonasliquifaciens* on the protein and DNA content and histopathalogical changes of head kidney *of Labeorohita.* Two experimental (Group-A, 10^{-2} + 10^{-2} CFU/fish; Group B, 10^{-3} + 10^{-3} CFU/fish) and two control (Groups a and b) groups of fish (66 in each group) were maintained for experimental purpose. Experimental groups (A and B) of fish received *A. liquifaciens* intramuscularly Six fish from experimental and control groups were sacrificed at hour 1, 3, 6, 12, 18, 24, 36, 48, 72, 96 and 216 of experimental period. The level of protein and DNA was found to be decreased throughout the infection period in both the experimental groups (A and B) when compared with their counterpart control groups (a and b). Head kidney showed inflammatory reaction, necrosis and glomeruli enlargement and congestion in the infected groups.

Key words: Aeromoniasis, protein, DNA, Head Kidney, *Ladeorohita*

INTRODUCTION

Fish infected with *Aeromonas* species may show numerous different clinical symptoms. These symptoms include lack of appetite, swimming abnormalities, pale gills, bloated appearance and formation of ulcers in skin and internal organs. Gills, kidneys, liver, spleen, pancreas and skeletal muscles are mostly effected by the oppurtunistic bacterial pathogens. The disease symptoms of aeromoniasis depend upon the virulance of the pathogen, resistance of fish to infection and environmental stress factors. The common occurrence of aeromoniasis in fresh water and marine water fish relates to the stress conditions or defense factors of the fish. Fish are easily stressed when they are mishandled, transported under poor water conditions and over crowded (Gupta etal.2008). *A. liquefaciens* may induce acute septicemic disease in both fresh water and marine water fish. (Azad, et al. 2001; Ashley, 2007) Nutrition and stocking density may also have a direct effect on certain biochemical indices in fish and the concentration of protein, cholesterol and glucose level is directly related to the sex, size and age of the fish. (Coz.-Rakovac et al., 2005, Dharansingh et al., 2008). Gupta *et al.* (2008) reported that juveniles of *Labeorohita*challenged with *A. hydrophila*showed moderately degenerated hapatocytes, oedema and leucocytic infiltration in parenchymatous tissues and extensive haemorrhage and haemosiderosis in the kidney. Yardimci and Aydin (2011) observed macroscopic and microscopic pathological changes in skin, gill, liver, kidney, heart, stomach, intestine, spleen, brain, eye and gonad of *Nile tilapia* (*Oreochromisniloticus*) at day 1, 2, 3, 5 and 7 of infection with *A. hydrophila*; the most significant pathological findings were observed in liver, kidney and heart. Parenchyma degeneration and necrosis of tubular epithelium in kidney and diffuse haemorrhage and lymphocyte infiltration in liver, kidney and heart were found in fish exposed to intraperitoneal injection of *A. hydrophila*.

The main objective of this study is to estimate the protein and DNA content, and to study the histopathological changes in the head kidney of *L. rohita* infected with repeated doses of *Aliquifaciens*

How to Cite this Article:
Satyalatha B.D.J and VivekaVardhani.V(2019).Aeromoniasis Induced Biochemical and Histopathological Alterations in the Head Kidney of *Labeorohita*.*Biolife*. 7(2), 9-12.
DOI:10.5281/zenodo.7403411

Received: 27March2019; Accepted: 18 May 2019;
Published online: 21May, 2019

MATERIAL AND METHODS

A total of 264*Labeorohita* (approximately weighing about 65-75 gm wt). aged 5-6 months were used in this study. The fish were divided into three groups each consists of 66 fish. Fish were treated with *A. liquefaciens* @ 10^{-2} +

10^{-2} CFU/fish (group A) and 10^{-3} + 10^{-3} CFU/fish (group B) at five days interval. Two groups (a,b) (66 each group) were kept as uninfected controls for comparison. All the fish were fed with pellet diet. The tissues of head kidney were collected and prepared for biochemical analysis and histopathology at hour 1, 3,6,12,18,24,36,48,72,96 and 216 of experimental period. Protein was estimated by the method described by Lowri et al. (1951) and DNA was determined following diphenyl amine method. Pieces of head kidney were fixed sectioned (5 µ) and stained by H and E method. Results were analyzed by student't' text to test the significance of protein and DNA level.

RESULTS ANDDISCUSSION

There was a marked decrease of protein and DNA content the head kidney of both the experimental groups (A and B) of fish when compared with controls from hour 1 to 216 of infection (except the slight of DNA on hour 1 and 6 in group B)

Protein Activity in Head Kidney(Table-1)

Infected fish of group A showed lower protein levels throughout the period of experimentation (from hour 1 to 216) when compared to that of uninfected controls. On hour 1 of infection, the head kidney showed a lower level of protein (58.96 mg/ml), which remained constant on hour 3 (58.27 mg/ml). The protein level decreased on hour 6 (55.51 mg/ml) and declined gradually upto hour 24 (51.37 mg/ml). Again there was no change in the level of protein on hour 24, 36 and 48 (51.37 mg/ml). There was a slight increase on hour 72, 76 and 216; however, these values are lower than control values. Experimental fish of group B, which received a lower dose of *A. liquefaciens*(10^{-3} + 10^{-3} CFU/fish) showed lower level of protein on hour 1 of infection (52.75 mg/ml) when compared to that of uninfected controls (63.79 mg/ml). From hour 1 to 18 (45.17 mg/ml) of infection, there is a gradual decrease of protein (these values were found to be below normal values). There was a slight increase of protein on hour 24 (56.89 mg/ml), 36 (57.58 mg/ml), 48 (57.93 mg/ml), 72 (55.86 mg/ml), 96 (57.58 mg/ml) and 216 (58.27 mg/ml). However, this decrease is lower than normal levels. It is clear from the results that the head kidney showed lower level (below normal level) of protein throughout the experimental period (from hour 1 to day 9).

DNA Activity in Head Kidney

In case of group A, which received infection (10^{-2} CFU/fish + 10^{-2} CFU/fish), the level of DNA content was lower than normals on hour 1 (10.0 mg/ml), 3 and 6 (7.77 mg/ml), 12 (6.66 mg/ml), 18 (5.55 mg/ml), 24 (4.44 mg/ml), 36 (1.11 mg/ml), 48 (5.55 mg/ml), 72 (4.44 mg/ml), 96 (5.55 mg/ml) and 216 (4.44 mg/ml). The content of DNA is below normal throughout the infection period. Experimental fish (group B) showed a higher level of DNA on hour 1 of infection (12.22 mg/ml). From hour 1 to 6 (15.55 mg/ml), there is a gradual increase – all these increased values are higher than that of control value (11.11 mg/ml) on hour 18 of infection. The DNA level decreased to normal level. From the hour 18 onwards till the day 9 of experimental period, a gradual decrease of DNA was observed (lower than that of control value).

Results were subjected to Student 't' test to find out the stastistical significance of protein and DNA level in the head kidney of different experimental and control groups of fish. Protein level in head kidney showed a significant rise in experimental groups A and B when compared with controls; and in between the experimental groups A and B (Table-2). The DNA level in head kidney showed a significant decrease in experimental groups A and B when compared with controls and in between the experimental groups A and B.

Table-1. Content of protein (mg/ml) and DNA (mg/ml) in the head kidney of experimental fish treated with *Aeromonasliquifaciens*10^{-2} CFU/fish + 10^{-2} CFU/fish (Group A) and with 10^{-3} CFU/fish + 10^{-3} CFU/fish (Group B) at different periods of infection and control (group a), (group b).

Hours of Necropsy	Experimental group		Control group	
	A		a	
	Protein	DNA	Protein	DNA
1	58.96	10.0	63.79	11.11
3	58.27	7.77	63.77	11.12
6	55.51	7.77	63.79	11.11
12	54.82	6.66	63.78	11.09
18	52.06	5.55	63.77	11.11
24	51.37	4.44	63.79	11.12
36	51.03	1.11	63.77	11.13
48	51.37	5.55	63.77	11.09
72	56.89	4.44	63.77	11.10
96	58.62	5.55	63.78	11.11
216	58.62	4.44	63.77	11.12
Hours of Necropsy	**B**		**b**	
	Protein	**DNA**	**Protein**	**DNA**
1	52.75	12.22	63.79	11.11
3	50.68	13.33	63.79	11.12
6	48.62	15.55	63.77	11.11
12	47.93	11.11	63.77	11.09
18	45.17	10.0	63.78	11.11
24	56.89	8.88	63.78	11.12
36	57.58	7.77	63.78	11.13
48	57.93	6.66	63.78	11.09
72	55.86	5.55	63.79	11.11
96	57.58	5.54	63.78	11.10
216	58.27	5.55	63.77	11.11

Values are expressed in the mean derived from five observations.

Histopathology:

Intramuscular injection of pathogenic bacterium caused much pathological changes like haemorrhages infiltration of lymphocytes, atrophy and necrosis throughout the

Table-2.'t' values obtained for different groups of fish infected with $10^{-2} + 10^{-2}$ (group A), and $10^{-3} + 10^{-3}$ (group B) CFU/fish

Experimental (A and B) and Control (a and b) groups				
	A	a	B	b
Head Kidney Protein				
Mean	55.22	63.78	53.56	63.78
	A a	B b	A B	
	|_____|	|_____|	|_____|	
t value	t=8.68* (P<0.05)	t=7.13* (P<0.05)	t=4.19* (P<0.05)	
Head Kidney DNA				
Mean	5.75	11.1	9.39	11.1
	A a	B b	A B	
	|_____|	|_____|	|_____|	
t value	t=7.64* (P<0.05)	t=2.75* (P<0.05)	t=3.05* (P<0.05)	

P value at 5% level of significance is 2.306 *Statistically significant values

infection period. In group A ($10^{-2} + 10^{-2}$ CFU/fish) the multiple sections of the head kidney showed heavy destruction and damage of entire tissue due to bacterial infestation. Tissue inflammatory reaction was evident by moderate increase of macrophages, lymphocytes and eosinophils. The blood vessels were highly congested. There is no evidence of ulcers or any proliferatory growth. Glomeruli sparseley congested (Fig. 1A). Fish which received double dose of infection ($10^{-3} + 10^{-3}$ CFU/fish; group B), the head kidney exhibited heavy necrotic tissue and severe blood clots. The tissue undergone heavy inflammation. Serous membranes of renal corpusles were showing proliferative inflammation. The hematopoietic tissue in the head kidney was disrupted by the heavy infestation of bacteria. Glomeruli were enlarged with heavy cellular proliferation (Fig. 1B). Vacuolar regeneration was seen in the renal tubule. Severe congestion of the renal capillaries was seen. The tissue undergone proliferation and hyperplasia. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed. The glomeruli showed nodular out growths due to severe bacterial infestation.

In this study, the pathogenic doses of $10^{-2} + 10^{-2}$ CFU/fish (group A) and $10^{-3} + 10^{-3}$ (group B) delivered orally induced a good secondary response particularly after the second booster dose. However, the reason for this secondary response in groups A and B is not known. It is possible that two multiple doses of infection are important in disturbing the level of proteins and DNA in muscle, gill, head kidney and brain.These studies are similar to those of Lamers and Van Muiswinkel (1986), Karunasagar*et al*. (1991); Ankamma et al (2017) and Newman (1993) who reported significant alterations in the profile of biochemical constituents using various antigen preparations of motile anomonads in fish. In the present study, *L. rohita*underwent stress due to the exposure of two varied multiple doses of *A. liquefaciens*. It is clear from the results of the present investigation that there were definite changes in protein fractions and their intensity profiles in head kidney at various days of infection. The stress caused by the aeromoniasis might have exerted ill effect in the organism at physiological tissue, cellular and molecular level as evident by the alterations in protein and DNA level (quantitative estimation), and histopathological reactions. These results confirm the observations of Begum (2004) who also observed biochemical alterations in liver and muscle tissue in fish, *Clariusbatracus*during insecticide treatment. The alteration of tissue protein level in head kidney might be due to the synthesis of stress proteins as reported by Welch (1993). Various authors (Boone and Vijayan, 2002; Tabche*et al.*, 2002; Ali *et al*., 2003) reported synthesis of stress proteins due to heavy metal treatment. In the present study, pathogenic aeromonads was found as effective inducer of stress protein in altering the tissue protein fractions of experimental fish (exposed to various doses of *A.liquefaciens)*. The histopathological changes in head kidney explain the involvement of stress in aeromoniasis; the changes like necrotic lesions and aggregations of melanin containing macrophages were observed in the present study. The pathological changes brought out in fish by pathogenic bacteria, strongly indicate that these lymphoid organs could provide sensitive indicators of stressful conditions in the aquatic environment. Fingerlings of *L. rohita*infected with *A. hydrophila*also indicated necrotic lesions and aggregation of melanine filled macrophages in kidney and spleen (Mohanty*et al*., 2008).

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Ali, K.S., Dorgai, L., Gazdag, A., Abraham, M. and Hermesz, E. (2003). Identification and induction of hsp 70 ene by heat shock and cadmium exposure in carp. Acta. Biol. Hung, 54(3-4): 323-334.

[2] Ankamma, N and VivekaVardhani, V(2017).Effect of dietary Aqua fix on qualitative protein profile in skeletal muscles of Labeo rohita.Biolife.5(2), pp279-283.doi:10.17812/blj.2017.5219

[3] Ashley, P.J. (2007) Fish welfare: Current Issues in aquaculture. Appl.Anim.Behav.Sci.104 : 199-235

[4] Azad, I.S., Rajendran, K.V., Raja, J.J.S., Vijayan, K.K. and Santiago, T.C.(2001) Virulence and histopathology of *Aeromonashydrophila* strains to tissue cultures. Microbios.77: 47-55

[5] Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clariasbatrachus*(Linn.) and recovery response. Aquatic Toxicol., 66(1): 83-92.

[6] Boone, A.N., and Vijayan, M.M. (2002). Constitutive heat shock protein 70 (HSC 70) expression in rainbow trout hepataytes: effect of heat shock and heavy metal exposure. Comp. Biochem. Physiol. Toxicol. Pharmacol., 132(2): 223-233.

[7] Coz-Rakovac R., Strunjak-Perovic I., Hacmanjek M., Topic PN., Lipez Z, and Sostaric B. (2005). Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrachuslabrax*) in the North Adriatic Sea. Vet Res Commun, 29: 677-687.

[8] Dharansingh. KamleshNath., Trivedi S.P. and Sharma Y.K. (2008) Impact of copper on haematological profile of freshwaterfish*Channa punctatus,* J. Environ. Biol. 29(2):253-257.

[9] Gupta, S.K., Pal, A.K., Sahu, N.P., Dalvi, R., Kumar, V. and Mukherjee, S.C. (2008). Microbial levan in the diet of *Labeorohita*Hamilton juveniles: effect on non-specific immunity and histopathological changes after challenge with *Aeromonashydrophila*. J. Fish Dis. 31: 649-657.

[10] Swapna Gurrapu and Estari Mamidala. In vitro HIV-Reverse Transcriptase Inhibition of Andrographolide Isolatedfrom Andrographis Paniculata. European Journal of Biomedical and Pharmaceutical Sciences. 2017. Volume 4, Issue 12. 516-522.

[11] Karunasagar, I., Rosalind, G. and Karunasagar, I. (1991). Immunological responses of the Indian major carps to *Aeromonashydrophila*vaccine. J. Fish. Dis. 14: 413-417.

[12] Lamers, C.H.J. and Van Muiswinkel, W.B. (1986). Natural and acquired agglutinin to *Aeromonashydrophila*in carp. *Cyprinuscarpio*. Can. J. Fish Aquat. Sci. 43: 619-624.

[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

[14] Mohanty, B.R., Mishra, J., Das, S., Jena, J.K. and Sahoo, P.K. (2008). An outbreak of aeromoniasis in an organised composite carp culture farm in India: experimental pathogenicity and antibiogram study. J. Aquaculture, 16: 27-37.

[15] Newman, S.G. (1993). Bacterial vaccines for fish. Ann. Rev. Fish Dis. 3: 145-185.

[16] Tabche, M.L., Gomes, O.L., Galar, M.M. and Lopez, L.E. (2002). Stress proteins produced by contaminated sediments with nickel in a pond with rainbow trout *Oncorhynchus mykiss* (Pisces: Salmonide). Rev. Biol. Trop., 50(3-4): 1159-1168.

[17] Welch, W.J. (1993). How cells respond to stress. Sci. Am. 268: 56-64.

[18] Yardimci Y.L. and Aydin N.L., (2011). Pathological findings of experimental *Aeromonashydrophila*infection in *Nile tilapia* (*Oreochromisniloticus*). Ankara Univ. Vet. Fak. Derg. 58: 47-54.