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COMPARISON OF BIOCHEMICAL VARIATIONS IN THE TISSUES OF PATHOGENIC AND NORMAL TISSUES OF Labeo Rohita

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ABSTRACT

Variations have been observed when pathogenic tissues and normal tissues have been compared for biochemical analysis, esterase and SDS protein studies. Proteins exhibited significant variation in liver and muscle. Whereas lipids exhibited variations in all tissues except liver and intestine. Esterase zymograms of gills and liver exhibited Loss of bands, hyperactivity of prevalent bands and induction of new bands in infected fishes. SDS proteins of liver in infected fish exhibited loss or reduced activity. The changes observed in protein, peptide and amino acid of the tissues in infected fishes, either increase or decrease, could indicate the presence of pathogens which secrete proteolytic enzymes and are also known to digest tissues.

Key words: Labeo rohita, Esterase, SDS Proteins, Pathogens, Proteolytic enzymes.

I. INTRODUCTION

Fishes being a rich source of protein are in great demand as food; they are also being used for different biomedical research. Normally fish are constantly challenged by pathogens and parasites; however stress, predators[1, 2] and pollution[3] could become the primary reason for pathogenicity and an epidemic. Comparisons between normal and healthy fishes are scanty at the biochemical level[4] reported higher haematocrit values in diseased silver carp[5] reported the variations in infected tissues leading to rise in metabolic flux of chemical constituents of tissues. In this work, comparative study of biochemical constituents of six normal and infected tissues muscle, eye, gill, liver, intestine and kidney were analyzed.

II.MATERIALS AND METHODS

The fish were caught from the ponds where infections and mortality were reported. Live fishes which exhibited inflammation of gills, were transported immediately to the laboratory which was near to the fish pond in water and then stored at cold temperatures. The various parts were visually observed for infections and were then homogenised suitably and analysed quantitatively for proteins[6], amino acids by colorimetric method[7] of, Lipids[8], qualitatively for esterases[9] and SDS Proteins[10]. Quantitative data were analysed statistically using ANOVA.

III. RESULTS

The total protein content, acid soluble peptides, free amino acids, lipids and amino acids were estimated in the tissues of normal and infected fishes. The results are consolidated in Table 1.

Total Protein Content: Muscle protein content was lower in infected fish (9.3%) when compared to that of normal fish (16.3%). In eye, gill and kidney there was not much of a variation. The values were higher in infected fish in liver (5%) and intestine (2.1%) when compared to the liver (2.5%) and intestine (1.1%) of normal fish. The values of muscle and liver were significant statistically.

Table 1: Biochemical Estimations of Tissues of Normal and Infected *Labeo Rohita*

(The values are expressed as Mean values ± SD)

Tissue	Proteins (%)*		Soluble Peptides (%)*		Free Aminoacids (%)*		Lipids (%)#		Water Content (%)*	
	N	I	N	I	N	I	N	I	N	I
Gill	4.50 ± 1.42	4.60 ± 3.19	0.59 ± 0.32	1.50 ± 1.14	4.25 ± 2.10	5.40 ± 2.26	26.0[a] ± 2.74	34.8[b] ± 3.28	30.3 ± 2.00	39.5 ± 1.55
Liver	2.50[a] ± 0.23	5.00[b] ± 0.30	1.00 ± 0.28	1.08 ± 0.47	6.80 ± 0.47	4.80 ± 2.36	85.7 ± 1.88	83.3 ± 3.14	75.0 ± 1.90	72.7 ± 1.06
Intestine	1.10 ± 0.50	2.10 ± 0.28	0.90 ± 0.28	0.71 ± 0.36	5.40 ± 1.60	3.60 ± 2.49	84.6 ± 1.69	88.8 ± 2.06	70.0[b] ± 1.07	56.6[a] ± 2.73
Muscle	16.30[b] ± 3.00	9.30[a] ± 0.11	0.52 ± 0.11	0.47 ± 0.16	4.50 ± 0.57	3.95 ± 1.72	25.0[a] ± 2.12	60.0[b] ± 5.14	71.4 ± 1.36	70.5 ± 1.58
Eye	4.30 ± 0.44	4.70 ± 0.61	0.81 ± 0.61	0.98 ± 0.43	1.30 ± 0.54	2.20 ± 1.79	75.0[b] ± 2.40	45.5[a] ± 2.64	54.3[b] ± 1.48	35.3[a] ± 1.89
Kidney	4.10 ± 1.35	3.80 ± 0.34	0.58 ± 0.25	0.55 ± 0.27	5.20 ± 1.64	4.50 ± 1.49	38.5[a] ± 3.27	50.0[b] ± 3.67	50.0 ± 2.55	46.6 ± 3.33

Note: * - mg / 100 mg wet weight, # - mg / 100 mg dry weight. The values with different (a, b) superscripts are significant ($p < 0.05$).

In case of acid soluble peptides and free amino acids, there was not much of a variation in all the tissues and the values were not significant, statistically.

Lipids: The lipid content estimated as dry weight of muscle (60%), gill (34.8%) and Kidney (50%) of infected fishes were higher as compared to the muscle (25%), gill (26%) and kidney (38.5%) of normal fishes. In liver and intestine there was not much of a variation. In eye of infected fish the percentage of lipid was lower (45.5) as compared to that of the normal (75.5%). The lipid content of gill, muscle, eye, kidney, of normal and infected fishes were found to be significant statistically.

Water Content: The water content of eye (35.3%) and intestine (56.6%) of infected fish had decreased in comparison to the eye (54.3%) and intestine (70%) of normal fishes. In gill of infected fish (39.5%) it had increased from the gill of normal fish (30.3). In muscle, liver not much of a variation was observed. Intestine and eye of normal and infected fishes were found to be significant, statistically.

Esterase Patterns: Zymograms of normal fishes had visible zones of esterases in gill with Rm 53.8, 32, 26, 15.4. Infected fishes, IF had different bands as compared to the normal fishes, the relative mobilities of the bands were varying and new but weak band with Rm 23 was noticed. In addition, visible band at 40, active band at 36.9, and hyperactive band at Rm 58.5 were present. . The above patterns are presented in Table 2 and Plate I.

Table 2: Gill Esterase Pattern of Normal and Infected *Labeo rohita*

Tissue	Rm							
	58.5	**53.8**	**40**	**36.9**	**32**	**26**	**23**	**15.4**
NF	-	+	-	-	+	+	-	+
IF	+++	-	+	++	-	-	±	-

The liver esterases of normal fishes had in all seven bands. The bands with Rm 10.8 had only traces of activity, visible bands at Rm 15.4, 26.2, 32.3, 46.2, 61.5 and active band at Rm 53.8. In case of infected fishes the activity of the bands were higher. The band with Rm 10.8 was hyperactive, other bands at Rm 15.4, 26.2, 32.3 and 58.5 were active. A comparison of the infected and normal fishes indicated that in infected fishes the band with Rm 10.8 was hyperactive, moreover it has lost three bands with Rm 46.2, 53.8 and 61.5. An Additional active band has developed at Rm 58.5. The esterase patterns of Liver are shown in Table 3 and Plate I.

Table 3: Liver Esterase Pattern of Normal and Infected *Labeo rohita*

Tissue	Rm							
	61.5	**58.5**	**53.8**	**46.2**	**32.3**	**26.2**	**15.4**	**10.8**
NF	+	-	++	+	+	+	+	±
IF	-	++	-	-	++	++	++	+++

Table 4 presents the pattern of esterases found in other tissues, viz. muscle, eye, intestine and kidney of *Labeo rohita*. There is not much of a variation between the infected and normal tissues. Some of the bands have exhibited either elevation or depression of activity. For e.g. Band with Rm 55.4 was active in the muscle of infected fish but the same band in kidney had shown a depressed activity in infected ones. The band with Rm 32.3 has reduced activity in kidney.

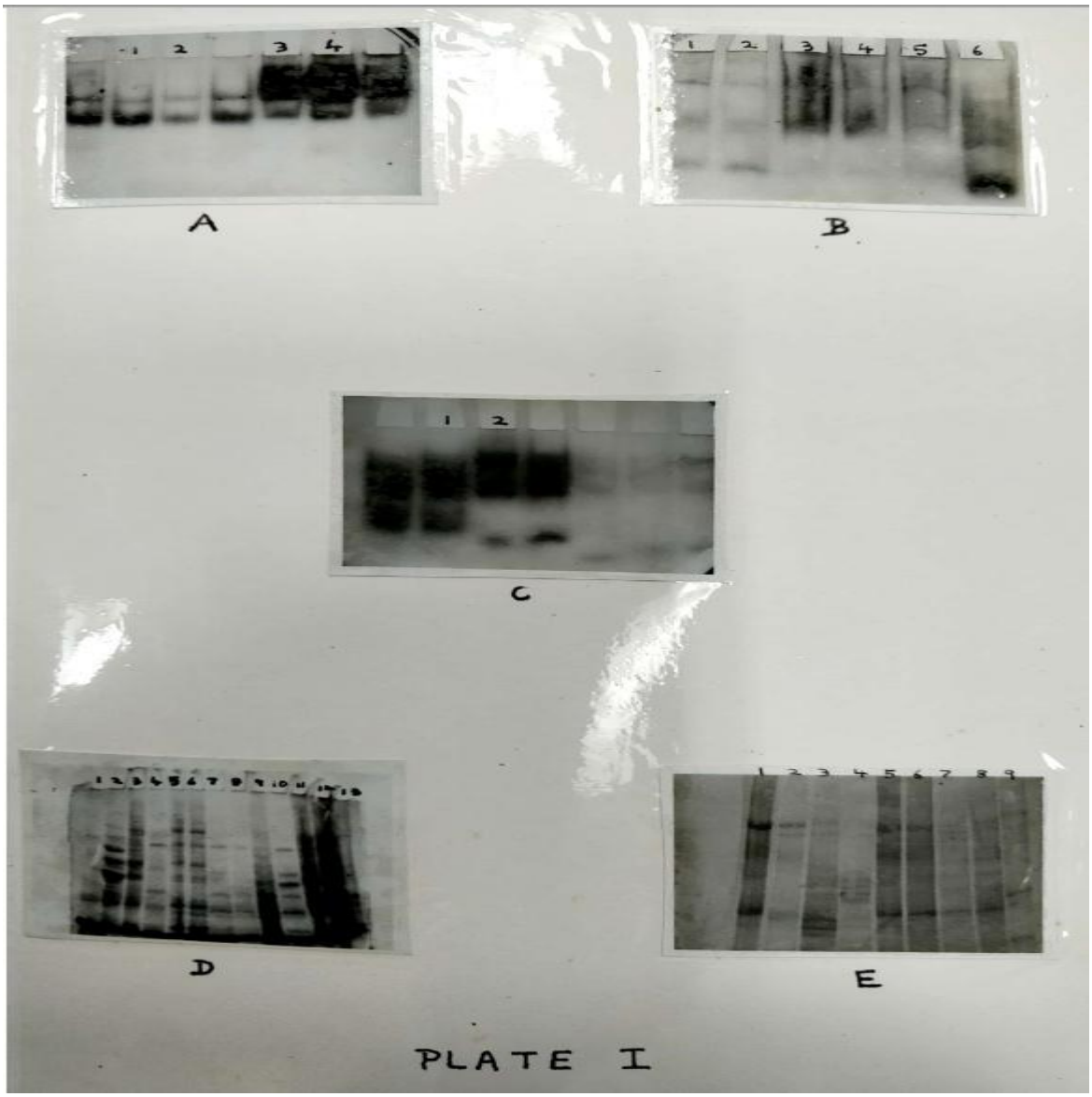

PLATE – I

ZYMOGRAMS OF ESTERASES OF NORMAL AND INFECTED TISSUES OF *Catla catla* AND *Labeo rohita*

A. Zymograms of Esterase of Gill and Liver of Normal and Infected *Catla catla*.

1⇒ NG = Normal Gill	2 ⇒ IG = Infected Gill
3 ⇒ NL = Normal Liver	4 ⇒ IL = Infected Gill

B. Zymograms of Esterase of Muscle, Eye and Gill of Normal and Infected *Labeo rohita*.

1 ⇒ NM = Normal Muscle	2 ⇒ IM = Infected Muscle
3 ⇒ NE = Normal Eye	4 ⇒ IE = Infected Eye
5 ⇒ NG = Normal Gill	6 ⇒ IG = Infected Gill

C. Zymogram of Esterase of Liver of Normal and Infected *Labeo rohita*

1 ⇒ NL = Normal Liver	2 ⇒ IL = Infected Liver

D. Zymogram of SDS Proteins of Tissues of Normal and Infected *Labeo rohita*

1 ⇒ NM = Normal Muscle	2 ⇒ IM = Infected Muscle
3 ⇒ NG = Normal Gill	4 ⇒ IG = Infected Gill
5 ⇒ NE = Normal Eye	6 ⇒ IE = Infected Eye
7 ⇒ NI = Normal Intestine	8 ⇒ II = Infected Intestine
9 ⇒ NL = Normal Liver	10 ⇒ IL = Infected Liver
11 ⇒ NK = Normal Kidney	12 ⇒ IK = Infected Kidney
13 ⇒ MW = Molecular Weights	

E. Zymogram of SDS Proteins of Tissues of Normal and Infected *Labeo rohita* and *Catla catla*

1 ⇒ NG = Normal Gill of *Labeo rohita*	2 ⇒ IG = Infected Gill of *Labeo rohita*
3 ⇒ NL = Normal Liver of *Labeo rohita*	4 ⇒ Infected Liver of *Labeo rohita*
5 ⇒ NG = Normal Gill of *Catla catla*	6 ⇒ IG = Infected Gill of *Catla catla*
7 ⇒ NL = Normal Liver of *Catla catla*	8 ⇒ IL = Infected Liver of *Catla catla*
9 ⇒ MW = Molecular Weight	

Table 4: Esterase Pattern of Muscle, Eye, Intestine and Kidney of normal and Infected *Labeo rohita*

Tissues	Rm							
	58.5	55.4	40.0	36.9	32.3	29.2	23.1	15.4
NM	-	+	-	+	+	-	-	+
IM	-	++	-	+	+	-	-	+
NE	-	±	-	-	+	-	-	+
IE	-	±	-	-	+	-	-	+
NI	++	-	-	-	-	-	-	-
II	++	-	+	-	-	+	-	-
NK	-	+++	-	-	+++	-	-	++
IK	-	+	-	-	++	-	-	++

SDS Proteins: In gills of normal fishes there are 14 bands. The slow moving band with Rm 26.6 and band with Rm 69.2 had stained more intensely (hyperactive). The bands with Rm 35, 37.5, 40, 43.3,51.6, 63.5, 75, 91.6 were visible bands in case of gills of normal fish. Three bands with Rm 30.8, 53.3 93.3 were present in traces in gill of normal fish. In infected fishes, bands with Rm 91.6 and 63.5 were visible, band with Rm 35 was absent and the remaining bands were stained in traces, the bands with Rm 53.3 and 3.5 had not stained and the remaining two bands were visible. An additional band had been inducted at Rm 70.8. The patterns of bands are as shown in Table 5 and Plate I.

Table 5: SDS Proteins of Gill of Normal and Infected *Labeo rohita*

Tissue	Rm													
	93.3	91.6	75.0	70.8	69.2	62.5	53.3	51.6	43.3	40.0	37.5	35.0	30.8	26.6
NG	±	+	+	-	+++	+	±	+	+	+	+	+	±	+++
IG	+	+	±	+	+	+	-	±	±	±	±	-	+	+

Liver of normal fishes has developed 13 bands of proteins. The bands with Rm 80.8, 77.5, 73.3, 62.5 had stained more intensely. In infected fishes the band with Rm 62.5 had the same intensity as that of liver of normal fishes. It had lost the bands with Rm 80.8, 56.6, 52.5, 40 and 35. In the bands with Rm 77.5, 73.5, 26.6, 30.8 and 34.8 there is a decrease in the intensity of staining and it had reduced to traces. Decrease in intensity of

staining was observed at Rm 26.6, 30.8, 34.8. The same intensity of staining was retained at Rm 87.5 and 91.6 as compared to the normal fish. An additional band of Rm 65 had been inducted. These patterns are presented in Table 6 and Plate I.

Table 6: SDS Proteins of Liver of Normal and Infected *Labeo rohita*

Tissue	Rm													
	91.6	**87.5**	**80.8**	**77.5**	**73.5**	**65.0**	**62.5**	**56.6**	**52.5**	**47.5**	**40.0**	**35.0**	**30.8**	**26.6**
NL	+	+	++	++	++	-	++	+	+	+	+	+	+	+
IL	+	+	-	±	±	+	++	-	-	±	-	-	±	±

In case of other tissues no variations of SDS proteins were noticed between normal and infected fish.

IV. DISCUSSION

The nature of damage to protein and lipid constituents, are the indicators suggesting the major turnover in protein and lipid systems of the tissues. The three fold increase in acid soluble peptides in gill with an enormous decrease in free amino acids and lipids is a significant feature. Reports on defense systems of fishes and amphibians[11,12] indicate that under the stress of infection, peptides are secreted by the epithelial linings of epidermis as well as other organs as trachea of bovine[13] and human small intestine[14]. No significant change has occurred either in the quantity of proteins and in the zymograms of SDS proteins, which further supports that peptides of low molecular weight might be synthesised *de novo* under infection. Much of the alteration recorded in protein, peptide and amino acid of the tissues in the fish is indicative of high protein turnover, either leading to depletion or synthesis of new proteins.

In the present study, in gills of infected *Labeo rohita*, an increase in acid soluble peptides was observed. Since defense peptides are quite often extracted in 1N acetic acid[15], it was concluded that gill too could have defense peptides which were secreted as a result of infection of the pathogens. Soluble proteins which include enzymes, hormones, free peptides, amino acids etc., play a major role in metabolism[16]. The changes observed in protein, peptide and amino acid of the tissues in infected fishes, either increase or decrease, could indicate the presence of pathogens which secrete proteolytic enzymes and are also known to digest tissues. The environmental conditions could be instrumental in variations observed in concentration of lipid[17]. Gill., which could change the hormone system[18.] Under toxic conditions gill damage occurs leading to decrease in chloride and increase in blood glucose and total protein[19] and impaired gas exchange.

The esterase pattern of the gill of the infected fishes did not show any variation in the number of bands. The activity of the enzyme (as evident by α-naphthol deposition on gels) increases substantially under infection. This was evident from the infection patterns observed.

In the liver elevation of activity of esterase bands were noticed. In addition fast moving zones of enzymes were induced under infection. Higher esterase activity has been reported in liver and stomach which are associated with digestion suggesting its role in digestion and metabolism of esters[20]. Esterases are among the enzymes

which respond quickly to the changes in environment[21]. Pollution of waters either by metals or pesticides were shown to increase the activity levels and also changes in the zymograms of esterases of fishes and other organisms.

Non-specific esterases were found to be excellent biochemical markers because their large numbers facilitated simultaneous analysis. These enzymes have been used to explore the normal and diseased mammalian central nervous system [22, 23.]

Aetiological agents of infection of Labeo rohita used in present investigation could not be isolated in pure forms. It is quite possible that under controlled conditions of aquarium waters the defense systems of fishes did not allow the pathogens to survive. fishes living in polluted waters are more susceptible for infection than the ones living in unpolluted waters[3]. Experiments repeated several times in the laboratory were similar to several experiments conducted and demonstrated by[24] both in field and laboratory condition.

In esterase patterns of liver in infected fish two bands have been lost, a new band had developed and there was an increase in activity in all the bands. This is supported by[25] who used esterases successfully as markers to study post surgery effects in glaucoma. SDS proteins of gill and liver and thin layer chromatography of lipids of infected *Labeo rohita* have shown variations including loss of bands and induction of new bands with deterioration in physicochemical conditions, it causes stress in fish leading to pathogenicity and mortality[26]. Isolation and study of lifecycle of pathogens and histopathological studies have to be undertaken to understand the pattern of pathogenesis. This could help in treatment of diseases and also play a pivotal role in increasing production in intensive culture of carps.

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