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A Comparative Study of Electrophoretic Patterns of Proteins in Different Tissues of Fresh Water Cat *Fish Heteropneustes fossilis (Bloch)*

Bheem Rao T

Assistant Professor, Department of Zoology, Kakatiya Government College, Hanamkonda
Email: thatipartibheemrao[at]gmail.com
Contact No: +91 9440720165

Abstract: ***The present study was carried out to determine the electrophoretic patterns of proteins in the gill, liver, intestine, muscle and brain tissues of freshwater cat fish H.fossilis (Bloch). Tissues were examined on 7.5% of SDS –PAGE. The protein patterns indicated that the gill and muscle have higher number of protein bands compared to other tissues. Total 10 number of protein bands observed in gill and muscle of test fishes, liver and intestine showed less number of protein bands i.e 7. The protein banding patterns were identified by standard marker protein and Rm values were calculated accordingly. The results of the present study of electrophoretogram of gill, liver, intestine, muscle and brain showed homology in protein bands with minor variations.***

Keywords: Protein patterns, SDS –PAGE, *H. fossilis*, Tissues

1. Introduction

Pesticide usage is a critical concern because it has an adverse effect on the delicate ecosystem. The transfer of the pesticides to the aquatic ecosystem creates a need to fully understand their effect in the resident biota.[1]. In many areas the sensitive ecosystems are at risk because of point source runoff pesticides from agricultural and urban sources to aquatic ecosystems affecting aquatic biota [2]. Pesticide pollution severely affects aquatic organisms and organisms at high tropic levels including human beings through food chain[3]. These pesticides absorb rapidly through different routes and accumulated in tissues like liver, kidney and fat and leads to many physiological and biochemical changes there by influencing the activities of several enzymes and metabolites and finally causes the disturbance of entire metabolic processes [4]. Many pesticides have been reported to produce a number of biochemical changes in fish and at lethal concentrations, pesticides have been attributed to enzyme and endocrinal activity as chemoregulators in fish.[5]. This may lead to provide an early warning signal to stressed organism. The source of these parameters is an indicator responding to environmental effects and can also serve as a marker for the xenobiotic exposure. [6 & 7]. In the present investigation an attempt has been made to study on electrphoretic patterns of proteins in gill, liver, intestine, muscle and brain of fresh water cat fish *H. fossilis* through SDS-PAGE.

2. Materials and Methods

Preparation of Samples for study at the end of each exposure period fishes were sacrificed, the tissues such as gill and muscle were dissected out and were used for the further analysis. The tissues were weighed to nearest milligram and were homogenized in 0.01M Tris HCl buffer (pH 7.5) containing 0.9% NaCl. The concentration of tissue homogenates varied from tissue to tissue. The tissues terhomogenation were placed in ice jacketed centrifuge tubes. The extracts were centrifuged at 2000rpm for 10min in a clinical centrifuge at room temperature. The supernatant were mixed with equal volume of 20% sucrose solution containing 0.5% bromophenol blue as tracking dye, An aliquot of 0.1ml of this mixture was used for loading the sample on to the gel for electrophoretic separation of protein patterns.

SDS-PAGE Analysis Homogenates (10%) of gill and muscle were prepared in Tris-HCl buffer (pH 7.2) and centrifuged at 10,000 rpm for 10min. The pellet was washed with chilled acetone and was dissolved in sample buffer 2ml of 0.5M Tris HCl (pH 6.8), 40% glycerol (1-6ml), 10% SDS (3.2ml), 2-mercaptoethanol (0.8ml), 0.1% (W/V) bromophenol blue (0.4 ml) and heated at 950c for 1min. Experimental procedure for preparation of SDS-PAGE The supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, β- mercaptoethanol and bromophenol blue was used as the tracking dye. An aliquot of 0.1ml (5mg) of the tissue extract was loaded on to the separating gel directly. The electrode buffer 0.025M tris and 0.192M Glycine was used for according to standard procedure [8]. whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with con. HCl. A constant current of 50 volts for the first 15 min followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8.0 cm from the origin. Staining Procedure and standardization of protein bands A solvent containing 0.25% Coomassie brilliant blue in methanol, water & acetic acid (5:5:1) was used for staining the proteins separated on gel by using standard method [9]. The molecular weight standards used in comparing the variations noticed in the SDS-PAGE, were of Low molecular weight protein standards (15 to 100 KDa) from the SIGMA-Chemical company (USA).

3. Results

Gill

The gill showed 10 protein bands with R_m values 0.23,0.41, 0.48,0.55, 0.61, 0.65, 0.70, 0.73 0.76 and 0.86. In these

three bands are in Zone-A(100-70KDa), four bands are in Zone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Liver

The liver showed 09 protein bands with R_m values 0.01, 0.05,0.23, 0.41, 0.48, 0.53,0.75, 0.86, 1.0; In this three bands are in Zone-A(100-70KDa),four bands are in Zone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Intestine

The intestine showed 07 protein bands with R_m values 0.25, 0.40, 0.50, 0.66, 0.75, 0.88 and 0.98. In this two bands are in Zone-A(100-70KDa), three bands are in Zone-B(55-35KDa), remaining two bands are in Zone-C(34-15KDa).

Muscle

The muscle showed 10 protein bands with R_m values 0.06, 0.26, 0.38, 0.46, 0.55, 0.70, 0.76, 0.80, 0.86 and 0.96. In this three bands are in Zone-A(100-70KDa), four bands are in Zone-B(55-35KDa), remaining three bands are in Zone-C(34-15KDa).

Brain

The brain showed 08 protein bands with R_m values 0.11, 0.23, 0.36, 0.50, 0.63, 0.73, 0.83 and 0.86. In this two bands are in Zone-A(100-70KDa),four bands are in Zone-B(55-35KDa), remaining two bands are in Zone-C(34-15KDa).

Table I: R_m values of all tissue protein patterns in fish *H. fossilis*

Standard Protein Marker	G	L	M	I	B
	0.01	0.01	0.01	0.05	0.01
		0.05		0.06	0.05
0.23	0.23	0.23	0.05	0.16	
0.38				0.21	0.15
	0.41	0.41			0.23
0.48	0.48	0.48			
		0.66			0.36
	0.53	0.53			
	0.58	0.83			0.50
	0.61	0.86	0.83		0.53
0.65					0.58
0.70					0.63
		1.0			
			1.0	0.66	
0.83	0.83	0.83			
0.86					
	1.0			0.76	1.0
				0.78	
				0.80	
				0.86	
				0.91	

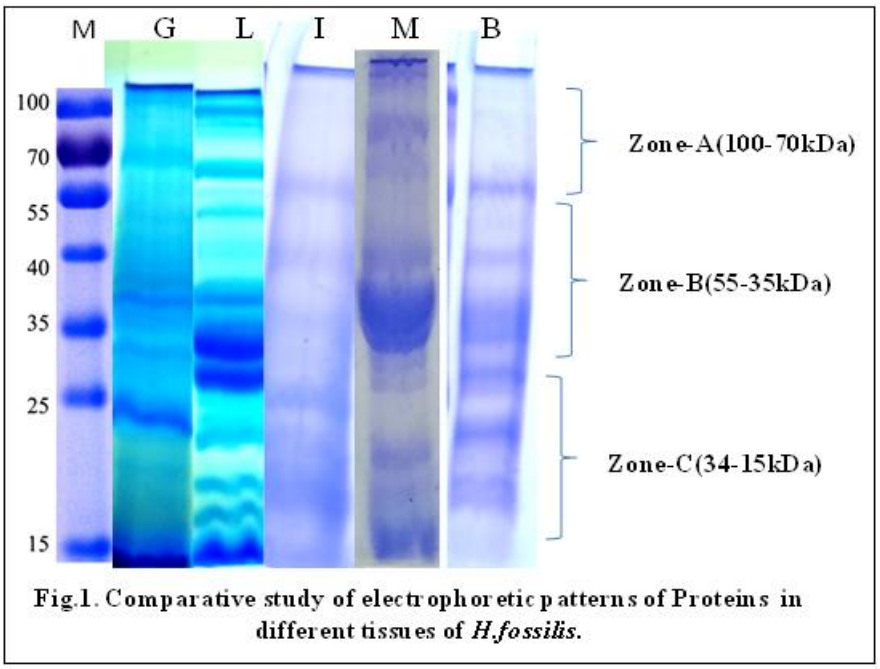
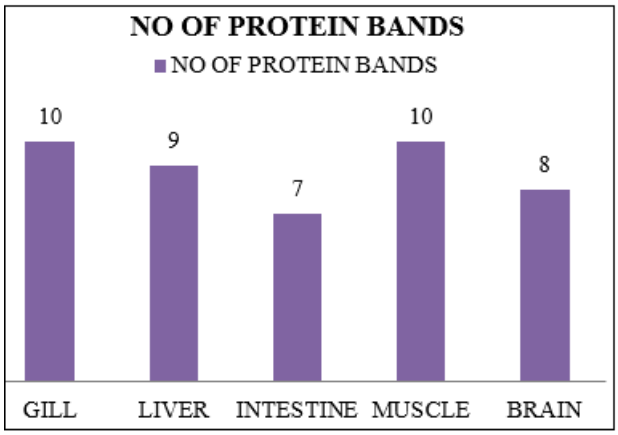


Fig.1. Comparative study of electrophoretic patterns of Proteins in different tissues of *H.fossilis*.



Graph 1: Graphical representation of total protein bands in different tissues in *H.fossilis*

4. Discussion

Electrophoresis, have been widely used in the classification of fish, these kinds of studies have brought about a new look to taxonomical evaluation [10]. Discrimination of related taxa can be easily made according to their electrophoretic results of serum proteins [11]. In the present study, electrophoresis of liver protein is used in discrimination of the three species studied. At present, there are number of taxonomical study on fish using SDS-PAGE techniques. In an investigation carried out by the serum proteins of *Capoeta trutta* and *Capoeta capoeta umbla* were analyzed by using SDS-PAGE. These investigators showed that there were 16 bands in *Capoeta trutta* and 11 bands in *Capoeta capoeta umbla*. They pointed out that *Cyprinus carpio* and *Pseudogobius esocinus esocinus* gave the smallest genetic distance.Many authors have reported similar observations, found the decrease in the protein sub units induced by Endosulphon and Fenvalrate in fresh water fish *Labeo rohita* through SDSPAGE. [12] The impact of Acetamiprid toxicity on electrphoretic patterns in liver, brain and gill tissues of fresh water fish *Oreochromis massambicus* [13]. had observed by using new techniques such as electrphoretic studies on the muscle proteins of three species of genus *Puntius* (Osteichthyes-Cyprinidae).

The protein subunits showed a steady decreasing trend in intensity of all the fractions throughout the exposure period demonstrating an inhibitory effect of Endosulphon on kidneyand muscle LDH.[. Jyothirmayi Tummala 2017; M.S. El-Sherif, et al 2009) have observed, that slight reduction (or) decrease in intensity of proteins in Diazinon treated fish Nile thilapia, which indicates that these proteins were highly affected the stress caused by the pesticide.(Kumar, K.B. and K.S. Devi 1992) have demonstrated that Malathion showed profound effect on protein patterns of *H. fossilis* and found new protein bands and some others disappeared after treatment. The electrophoretogram of gill,liver, intestine, muscle and brain showed homology in protein bands with minor variations. SDS-PAGE technique as biomarker for fish toxicological studies (Ola I. Muhammad et al, 2021). Proteins, the important constituents of animal tissues play a significant role in spare energy. These are the primary effector molecules of all living systems and any adaptive responses to environmental, physiological (or) pathological conditions will be reflected by alterations in protein activity (VJ Florence Borgia et al., 2019). The pesticides may inhibit the expression of some genes (or) activate the others to produce specific mRNAs which may subsequently be translated into specific proteins called stress induced proteins. An alteration of protein metabolism was observed in fish exposed to various types of environmental stress like materials and pesticides (Swetha and Gopal 2009; Alexandria et al., 2009). The appearance of new protein bands at different time intervals after exposure of the pesticide demonstrated clearly the alterations in the cytoplasmic protein patterns (Tripathi and Sukla 1990a; 1990b); (Justin Raj et al., 2017). In the present study, changes were observed on SDS-PAGE performed for the tissues of brain, intestine and liver of fish H. fossilis exposed to Methyl parathion, when compared to control. The protein subunits of pesticide exposed tissues showed decreased in the intensity and some protein bands have disappeared. The proteins showed more decrease in intestine (or) significant fading in Methyl parathion exposed tissue samples. Many authors reported that the similar observations (Veeraiah et al., 2014) were observed the appearance (or) disappearance of some proteins fraction in the tissues of different fishes like H. fossilis. Badaway et al., 1998, reported the electrophoretic serum proteinograms of Clarias gariepinus. The Fenvalrate induced toxicity on digestive enzyme such as proteinase and amylase of Zebra fish (Justin Raj and Baby Joseph 2014), reported the impact of textile dyes and Acetamiprid toxicity on electrophoretic patterns in liver, brain and gill tissues of fish Oreochromis mossambicus, Jyothirmayee et al., (2006) studied the alterations in the serum electrophoretic profile of the edible fish Anabas testudineus and Clarias batrachus The differences of proteins between species is specific for individuals representing a group. This could elucidate taxonomic problems in the case of dispute species (smith *et al*., 1979). In electrophoretic technique, closely related species share many electrophoretic alleles but also differ at some which they are fixed for different alleles (Smith *et al*., 1990). If two different species have same number of electrophoretic fractions, further close comparison of relative mobility of one or more bands could reveal well-defined species-specific differences. Comparative study of profenofos and carbosulfan clearly indicate the toxic nature of both pesticides on protein fractions in different tissues of *Labeo rohita* and the profenofos may be more effective than carbosulfan to non-target organism such as fish. (Bantu Nagaraju, *et al*., 2016). The electrophoresis technique is one of the important tool to study the protein pattern to distinguish species diversity and genetic differences in fin fish and shellfish (Mohana Rao *et al*., 2016). Similar results were found in Sherif *et al*., 2009; Sipra mohapatha *et al*., 2012 clearly reported that alterations in the cytoplasmic proteins, the appearance of new protein bands after exposure to pesticide. Effect of Insecticide on fish exhibits different modes of action and seem to be tissue specific (Veeraiah, K *et al*., 2014). Long term exposure to acetamiprid means a continuous health hazard for the fish population (Justin Raj and Baby Joseph, 2017).Pesticide causes remarkable disturbance in protein profile of fishes (Vishal Rajput and Richa Gaur, 2015). Long term exposure of Methyl parathion becomes a continuous health hazard for the fish population. Therefore it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish (Bheem Rao *et al*., 2018) The electrophoretogram of

both gill and muscle of *H. fossilis* showed homology in protein bands withminor variations. Electrophoresis cannot demonstrate the identity of two proteins; it can only show differences. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique widely used in biochemistry, genetics and molecular biology to separate proteins according to their electrophoretic mobility. In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes often has been used by some researchers as an aid in the species identification of fish (Miyazaki J.I., Hirabayashi T., Hosoya K., Iwami T.A,1998; Focant B., Jacob M.F., Huriaux F.1981; Pineiro C., Vazquez J., Marina A.I., Barros-Velazquez J., Gallardo J.M,2001). A Comparative Study on Total Muscle Protein Content of Anabas testudineus, Labeo gonius, Labeo rohita and Heteropneustes fossilis and to analyze their Electrophoretic Banding Pattern using SDS-PAGE (Dipika Doloi et al,2020).

5. Conclusion

Accordingly, to the results which mentioned for the first time using SDS-PAGE for some Red Sea fishes, we can conclude that, researchers can use protein profile not only in taxonomy, populations relationship but also to study the toxicological aspects of those species along the Red Sea in some polluted areas. The alterations of protein bands observed in different tissues of treated fish, the differences of proteins between species is specific for individuals representing a group. This could elucidate taxonomic problems in the case of dispute species. In lectrophoretic technique, closely related species share many electrophoretic alleles but also differ at some which they are fixed for different alleles. If two different species have same number of electrophoretic fractions, further close comparison of relative mobility of one or more bands could reveal well defined species specific differences

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