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Research Paper

Modulation of Serum Proteins by Methyl Parathion in the fish Labeo rohita and Cirrhinus mrigala

Ecotoxicology

KEYWORDS : Serum protein fractions, methyl parathion, immune response

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ABSTRACT

The fish as an indicator species plays an important role in the monitoring of water pollution because it responds with great sensitivity to the changes in the aquatic environment. Alterations in the serum protein fractions in response to pesticidal stress (methyl parathion) were investigated in Labeo rohita and Cirrhinus mrigala in the present study. The fish serum proteins showed 5 distinct bands viz. albumin, α1, α2, β and γ. The results showed that the α1 and α2 increased exponentially till 96 h in Labeo rohita whereas γ- globulin increased in Cirrhinus mrigala suggesting that innate response was more in Labeo rohita and acquired immune response more in Cirrhinus mrigala. The results also suggested that immune response could be altered and modulated by methyl parathionwhich could be due to the direct and indirect interactions with the corresponding genes in Labeo rohita and Cirrhinus mrigala. The physiological and biochemical significance of the serum protein profile following the treatment of methyl parathion is discussed herein.

Introduction:

Toxic substances may be introduced deliberately or accidentally into the aquatic ecosystem impairing the quality of water and making it unsuitable for aquatic life. When the concentration of the toxic substance is higher than what the homoeostasis of the aquatic organisms can sustain, it results in death or organ damage. In fish, organs such as the opercula, the liver, the kidney and the gill could be impaired (Oyedapo and Akinduyite, 2011). A toxic substance is a chemical pollutant that is not a naturally occurring substance in aquatic ecosystems. The greatest contributors to toxic pollution are pesticides and industrial compounds such as cyanides, nitrates, heavy metals, etc (Agarwal et al, 2010). The entry of the toxicants in the aquatic media may affect the water quality parameters which in turn leads to changes in the haematological variables of the fish due to its close association with the external environment (Carvallo and Fernandes, 2006; Kavitha et al, 2010). Monitoring of blood parameters, both cellular and non- cellular may have considerable diagnostic values in assessing early warning signs of pesticidal poisoning.

Blood is a pathophysiological reflector of the whole body. Knowledge on the physiological action of the toxicant helps to predict an important sub- lethal effects and analysis of biochemistry, haematology and histopathology may be used to determine the mode of action of the toxicant. Thus, the mechanisms of the fish adaptations to environmental conditions could be associated with the specificity of the metabolic status. Serum proteins are the complex of multifunctional system which may reflect the metabolic level of the organism and its adaptations to the environmental fluctuations (Mc Donald and Milligan, 1992; Zowail et al, 1994). Serum albumin is the major protein of the blood of the human beings which play an important role in the transport of wide range of physiological exogenous ligands and regulation of the colloidal osmotic pressure of the blood (De Smet et al, 1998). On the other hand, fish albumin also has phylogenetic significance. In some elasmobranchs it was not shown, among teleost fishes several species have been reported to be absent. In few species, specific properties of albumin were observed and they were identified and called albumin like proteins (Hasnain, et al, 2004). Besides both, fish physiological status and environmental factors impact albumin and may change its characteristics depending on fish ecological status (Lukyaneko and Habrov, 2005), chemical toxicants (Richmonds, 1990) and long term pollution of habitats (Moassa et al, 1994; Sayeed et al, 2011).

Fish are also particularly good models for studies in which biochemistry and comparative physiology are involved because they live in diverse habitats and must adapt to environmental parameters and stress, both of which can easily be reproduced under laboratory conditions. The understanding of toxicants uptake, behaviour and responses in fishes, therefore has a high potential for ecological relevance.

A recent proteomic study has identified upto 81 components in human plasma (Park et al, 2006). However, investigators generally relied on electrophoretic co-migration of components of sera under investigation with well characterized fractions of mammalian serum (Mallion and Himmo, 1993; Thurston, 1967) while due to prime role in immune response, much of the recent attempts on fish plasma proteins continue to focus on immunoglobins (Hasnain et al, 2004). Identification and characterization of fish serum albumin has been reported by Jabin and Husnain, 2001.

From evolutionary perspective, quantitative and qualitative status of several well identified abundant proteins in lower vertebrates need explanations. For instance, in some fish species albumin may exist in minute quantities, while sera of other fish species may be entirely lacking (De Smet et al, 1998).More primitive hagfish contain a large sized lipoprotein that binds molecules typically transported by vertebrate serum albumins (Gray and Doolittle, 1992).

The proteins occupy a unique position in the metabolism of the cell because of the proteinaceous nature of all enzymes which mediate at various metabolic pathways. The serum proteins of the fish have been occasionally studied in the fish to estimate the toxic potential of many substances including pesticides. Organisms may respond to the toxicants with up or down regulation of the serum proteins which could give a view of the defence pattern against them. These changes are important in assessing the impact of exposure under natural conditions and may also serve as tools for biological monitoring. The development of electrophoretic techniques makes it possible to detect the protein compositions. The potential value of electrophoresis in this study is based on the hypothesis that stress conditions may cause significant quantitative and qualitative changes in the proteins exposed to pesticides. Such changes may reflect an altered antibody synthesis, protein synthesis, cellular leakage or

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perhaps other events resulting directly or indirectly from stress. However, very scanty reports are available on variations of qualitative and quantitative serum proteins, especially with reference to the pesticide, methyl parathion in the most popular edible fishes Labeo rohita and Cirrhinus mrigala. As such, the aim of the present study is to look into the protein profile of the serum in the fish Labeo rohita and Cirrhinus mrigala as a function of methyl parathion by using automatic electrophoresis and densitometry.

Materials and Methods:

Experimental fish: Healthy and active specimens of fish Labeo rohita (Rohu) and Cirrhinus mrigala(Naini) selected as models for the present investigations, were procured from local hatcheries and brought to the laboratory and kept in stock aquarium. The mean weight of the fishes was about 90 g.

Maintenance of animals: The fishes were transferred to the stock aquarium of the laboratory containing tap water for about a week so that they became acclimatised to the laboratory conditions. The water was changed every alternate day. Fishes were fed properly with commercial feed composed of ground shrimps available in the local market to avoid the effects of starvation. The physico- chemical characteristics of the aquarium water were measured (Tab. 1).

Determination of LC_{50}: The experiment was repeated several times and only arithmetic mean of the experiments at each concentration was taken to express the results. LC_{50} values were determined by EPA Probit Analysis Programme (Finney, 1971).

Blood collection: The fish was caught individually with small hand net from the aquarium with minimum disturbances. After preliminary investigations of the length and weight, the blood samples were collected from the caudal fin as described by many authors but in the present investigation the collection of the blood had to be abandoned because there was a marked increase of the enzyme activities mainly of creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) which leaked from the surrounding muscles. Thus, cardiac sampling was the only suitable method of obtaining blood under study. The collected blood was immediately transferred to the eppendorf tube and left for 15- 30 minutes at room temperature for the clot formation. The clean non- hemolyzed serum was centrifuged in a cooling centrifuge (Model- C-30) at 4^0C for 10 minutes. Thereafter, the serum was transferred to another eppendorf tube for serum electrophoresis.

Separation of Serum Proteins: Among the various methods available for separation of proteins, electrophoresis is a well established and versatile technique, widely used in clinical chemistry. In the present study, the serum protein was separated in Interlab Pretty, a fully automatic electrophoretic instrument using agarose gel plate at pH 8.6. The gels were stained in acid blue and destained by aqueous solution of citric acid and then washed by aqueous solution of surfactant. All these solutions were placed in appropriate containers which were connected to the electrophoretic unit by piping system. The staining and destaining were done in a proper sequence already programmed in a driver software system. Quantification of the dye colour intensity associated with the bands was accomplished by scanning the slide in a measuring optical system (densitometer) inside the instrument. The graphs obtained by Elfolab software elaboration of the densitometer and the data displayed the percentage value of each fraction. The area of each profile fraction was computed as a percentage of the total protein. Comparisions were then made among the protein profile on the basis of these percentages for correspondingly numbered fractions. The absolute value of each band reported in g/l was calculated by multiplying the percentage value by sample's total protein concentration. The total concentration of serum protein was determined by Biuret method (Gronell, et al, 1949). Fish serum along with human serum was run simultaneously for easy comparison with that of human.

Results and Discussion:

Different serum protein fractions in both the fishes under study migrated from the origin quite similar to that of human while albumin was the major protein of the human sera, its co- migratory band existed in much lesser amount in fish Labeo rohita and Cirrhinus mrigala. The changes in the total and different fractions of proteins in the serum of any organisms are good indicators of ecological stress, physiological homeostasis and aquatic pollution. Most of the authors have viewed proteins as an alternative energy source but their roles in immune responsiveness in the fish blood under toxic conditions have been scantly reported. Serum proteins can be used to detect the fish health (De Pedro et al, 2005) and play a crucial role in immune response. The basic function of the immune system is to protect individuals against infectious agents, pathogens and toxicants. Proteins are the most abundant component in serum and are used in forming defensive molecules that help the body to fight against infections and xenobiotics. There are mainly two groups of serum proteins, albumins and globulins. Albumins are very good predictor of health and important for tissue growth and also as carrier proteins whereas globulins play an important role in defence mechanisms.

In the present study, the fish serum proteins have been identified with respect to the human serum proteins because the later have been exhaustively studied. The human serum, the pattern from cathode to anode showed 5 distinct bands which were as: albumin, α_1, α_2, β and γ- globulins. The fish serum has also shown 5 bands and the first band of the fish serum protein was in the close proximity to the first band of the human serum protein whereas the rest of the bands migrated slightly differently than the human serum proteins (Fig.1). The total serum proteins, albumin and α_1, α_2, β, γ- globulins in the control C. mrigala were higher than L. rohita. Relative electrophoretic mobilities of serum proteins of Labeo rohita and Cirrhinus mrigala suggested slight homology with those of human serum. There was however slight species specific differences in the relative electrophoretic mobilities and quantities of the identified serum proteins of Labeo rohita and Cirrhinus mrigala suggesting phylogenetically to be similar. Following the exposure of fishes to methyl parathion, there were changes in the band intensity which could be interpreted as a result of certain mutational events that would have occurred in the regulatory genes which could lead to inhibition, alteration or constitutive gene expression. As such, the corresponding bands became more intense or faint (Fig.1). The data presented in Tab.2 showed the changes in serum total protein, albumin and globulin levels of control as well as fishes subjected to exposure of sub- lethal dose of methyl parathion. Evidently, the total serum protein in Labeo rohita and Cirrhinus mrigala increased (Tab.2) following the treatment of methyl parathion. Ray et al, 2015 reported that following the treatment of the pesticide, the liver showed expanding sinusoids, necrosis and vacoulation of hepatocytes showing increasing hepato- cellular damage resulting in the leakage of proteins into the blood and thereby increasing total serum proteins. The serum protein electrophoresis revealed high differences between the control and the treated fishes. Tab.2 also showed that while albumin was the major protein of human sera, its co- migrating band existed in much lesser amount in both the fishes under study. Albumin was typically the major anionic protein in the vertebrate plasma representing more than 52% of the plasmatic protein. It has an important role in transport of endogenous ligands and xenobiotics mostly through the formation of non- covalent complexes at specific binding sites. The values of albumin concentrations were much lower in fish than human. Since fishes formed the largest group among the vertebrates with wide variability in

shape, size, anatomical and metabolic characteristics, physiologically such variability reflected the great capacity of adaptations to the aquatic environmental diversity. Albumin in fish participated in plastic metabolism and performed transport functions of substances primarily all lipids as well as xenobiotics for life sustainability (De Smet, 1998; Baker, 2002). Fish consumed xenobiotics with food and water which bound with albumin and changed its electrophoretic mobility (Osman et al, 2010).

Following the treatment with methyl parathion there was an initial decrease in 24 and 48 h of albumin and thereafter sharpincrease in albumin concentration in 72 and 96 h (Tab.2). The restoration of albumin, after initial decrease, after 48 h of exposure there seemed to exist an oscillatory phase of protein turn over towards more synthetic phase leading to recovery and adaptation phenomena.

α_1 and α_2 globulins in both the fishes under study migrate from origin quite similar to that of human (Fig.1). Both the fractions come under CRP (Complement Reactive Protein). CRPs were the component of the innate immune system responding against toxicants and pathogens. The concentration of α_1 globulin in control group of Labeo rohita and Cirrhinus mrigala were 8% and 9.5% respectively, whereas the concentrations of α_2Labeo rohita and Cirrhinus mrigala were 56.7% and 54.0% respectively. It was interesting to note that following the treatment, there was a marked increase in α_1 in Labeo rohita till 96 h whereas in Cirrhinus mrigala there was an increase till 48 h and thereafter a decrease in 72 h and 96 h. It was quite evident from the result shown above that the speed with α_1 and α_2 increased in Labeo rohita and Cirrhinus mrigala was remarkable being faster than could be accounted for by established mechanisms of shock response. In Labeo rohita the increase of α_1 and α_2 was till 96h. Ray and Sinha (2014) have reported that cortisol increased significantly after 2 h following the treatment of sub- lethal dose of methyl parathion. In the present study, α_1 and α_2 also increased sharply following the treatment. This supports the idea that constitutive internal defences are potentiated when the earliest stress hormone increased in the serum (catecholamines) to prepare the fish for "fight and flight" response.The increase in α_1 and α_2 globulins could be a part of the basis for the enhanced acute phase response involving systemic and early non- specific defence mechanisms. On the contrary in Cirrhinus mrigala there was a remarkable increase of γ- globulin following the treatment of methyl parathion till 96h. The observed increase of γ- globulin in Cirrhinus mrigala could be responsible for the increased production of immunoglobulins (IgM). This could be considered as specific immune response by way of immunoglobulins working as antibodies in the opsonisation process against methyl parathion. Similar reports have been made by Magnadottir and Gudmundsdottin (1992).

Conclusion:

Fishes are the first vertebrates to have both innate and adaptive immune mechanisms similar to mammals. Thus, fish can be used as a model in biomedical research, allowing data in the immunotoxicology field in evolutionary terms. Besides, the fishes being the most abundant vertebrates on the planet earth, a lot of them with commercial importance, data generated could have economic and ecological importance.

The results suggest that the immune responses could be altered by methyl parathion exposure. The significant modulation by the pesticidal stress suggests that methyl parathion might have direct or indirect interactions with the corresponding genes in Labeo rohita and Cirrhinus mrigala. As these biomolecules are key regulatory elements such changes induced by methyl parathion might have serious bearing on the physiological fitness of the two fishes under study. Alterations in the serum protein fractions could serve as a sensitive biochemical indicator of methyl parathion pollution in the aquatic environment which might help in water quality control and management as well as fish productivity with respect to the indiscriminate impact of methyl parathion from agricultural sites.

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Tab.1

Physico- chemical characteristics of aquarium water

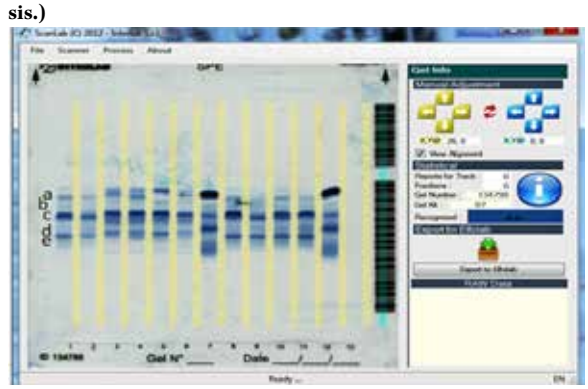
Sl no.	Parameter	Values
1	Temperature	30^{0}C
2	pH	7.5
3	D.O. (mg/l)	7.0
4	Electrical conductivity (μs/sec)	350
5	Total hardness (mg/l)	300

Tab. 2

Serum protein fractions percentage of Labeo rohita (Rohu) and Cirrhinus mrigala (Naini)

	Albumin %		α1 globulin%		α2 globulin%		β globulin%		γ globulin%	
Duration	Rohu	Naini	Rohu	Naini	Rohu	Naini	Rohu	Naini	Rohu	Naini
Control	11.5	12.3	8.0	9.5	56.7	54.0	11.8	12.9	12.0	11.3
24 h	8.8	8.2	19.2	36.6	54.6	32.6	10.7	12.2	6.7	11.4
48 h	8.8	6.4	20.4	15.9	53.8	41.1	4.9	19.0	7.1	17.6
72 h	14.9	10.0	15.4	8.8	55.2	49.0	7.4	11.8	7.4	20.4
96 h	14.5	7.1	18.9	6.3	49.3	47.0	9.1	16.0	8.2	23.6

Fig: 1 Electrophorogram of Serum Proteins (Gel Info. Panel showing: Manual adjustment for aligning the bands from cathode to anode, Statistical: showing Gel ID, Export to Elfolab: showing preparation of the software for data analysis.)



Legends:

a=Albumin; b= α_1 globulin; c= α_2 globulin; d= β globulin; e= Yglobulin, for all the lanes from 1-13;

1-13 are lanes of samples of serum representing as follows:

Lane no.	Serum sample name
1	C. mrigala (Naini/Control)
2	L. rohita (Rohu/Cntrol)
3	Rohu/Methyl Parathion Treated/24 h
4	Rohu/Methyl Parathion Treated/48 h
5	Rohu/Methyl Parathion Treated/72 h
6	Rohu/Methyl Parathion Treated/96 h
7	Humam/Nornal
8	Naini/Methyl Parathion Treated/24 h
9	Naini/Methyl Parathion Treated/48 h
10	Naini/Methyl Parathion Treated/72 h
11	Naini/Methyl Parathion Treated/96 h
12	Human/Normal
13	Empty

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