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ORIGINAL RESEARCH ARTICLE

Fourier Transform Infrared Spectroscopic (FT-IR) Study on Arsenic Induced in Gill Tissue of Freshwater Fish, *Ctenopharyngodon idella*

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ABSTRACT

Arsenic are well-known pollutants in the aquatic environment, pose a serious environmental hazard because of their persistence and toxicity. Arsenic produce biochemical changes in the organs of animals due to their potential toxicity. In the present study, the effect of arsenic induced biochemical changes in the gill tissues of freshwater fish, *Ctenopharyngodon idella* using Fourier Transform Infrared (FT-IR) Spectroscopy. The FT-IR spectrum of gill tissue is quite complex and contains several bands arising from the contribution of different functional groups belonging to lipids, proteins, nucleic acids and glycogen. The present FT-IR investigations, it has been observed that in the gill tissues, the amount of protein contents were found to be decreased whereas, the amount of lipid content were decreased and/or increased in the gill tissue region than, the control fish, may be suggested due to the exposure of arsenic after 28 days, respectively. In conclusion, the present study shows that, the gill tissues are vulnerable to arsenic intoxication. The result further suggests that, the arsenic intoxication induces significant alteration on the major biochemical functional constituents such as lipids, proteins and nucleic acids, which can be easily evidenced by FT-IR spectroscopy.

Key words: FT-IR, arsenic, *Ctenopharyngodon idella* gill tissue, biochemical changes.

1. INTRODUCTION

Arsenic is one of the heavy metals that occurs naturally in water, soil and air. Arsenic may undergo a variety of reactions in the environment including oxidation-reduction reactions, ligand exchange, precipitation and biotransformation [1]. Arsenic belongs to the group of highly toxic heavy metal indeed, long term exposure of arsenic induced impairment of reproductive function and neuroendocrine disruption. Currently accepted opinion of arsenic action is related to proteins synthesis and enzyme functioning [2].

Accumulation of arsenic in the body leads to physiological and biochemical disorders. An aquatic ecosystem is a diverse assemblage of organisms and biotic factors, which are inter related, even the micro-changes in the environment can later the nature and behaviour of the organisms. Freshwater fish constitute one of the major sources of cheap nutrition for the human beings. The nutritional values of fish depend on their biochemical composition like proteins, carbohydrates, free amino acids, lipids and mineral contents. It is known that the tissue proteins, carbohydrates and lipids play a major role as energy precursors for fish exposed to stress conditions. The majority of toxic substances initiate biochemical alterations like, inhibitions of enzyme system, alteration in the level of enzyme and specifically alteration in the permeability of biological membranes [3].

Proteins are highly sensitive to heavy metals and are one of the earliest indicators of the heavy metals poisoning. The impairment in protein synthesis due to heavy metal stress was reported by many investigators [4,5]. Lipids are an important constituent of animal tissue and play a prime role in energy metabolism. Lipids are also important in cellular and sub-cellular membranes. Carbohydrate is an essential energy source for all vital activities of organisms. Glycogen is the stored from of carbohydrate and plays an important role in carbohydrate metabolism.

Fourier Transform Infrared Spectroscopy (FT-IR) has recently emerged as one biomedical technique that can potentially reveal a wealth of qualitative

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and quantitative information about a given biological or cellular sample [6]. The increasing use of FT-IR spectroscopy demonstrate that, this technique is a valuable groups belonging to tissue components, such as membranes, proteins, nucleic acids, polysaccharide components as well as for complex biological materials such as tissues, body fluids or cell cultures. The shift in the peak positions, band width and the intensity of the bands, all give valuable structural and functional information which may have diagnostic value for biological systems. Therefore, it seemed interesting to apply FT-IR spectroscopy to monitor disease induced and arsenic toxicity induced biochemical changes occurring in any living organisms and to evaluate the quantitative and semi quantitative effects of various agents on biological structures.

FT-IR spectroscopy, it makes possible to simultaneously monitor changes in the structure and properties of biomolecules such as proteins, lipids and carbohydrates in biological tissues and cells [7,8]. The present study was undertaken to investigate the effect of arsenic trioxide on the biochemical contents of functional groups in gill tissue of the freshwater fish, *Ctenopharyngodon idella* after 28 days exposure periods by Fourier Transform Infrared Spectroscopy study.

2. MATERIALS AND METHODS

The freshwater fish, *Ctenopharyngodon idella* having mean weight 16-18 gm and length 14–16 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1%KMNO4 solution and then kept in plastic pools for acclimatization for a period of nine days. They were fed on rice bran and oil cake daily. The arsenic trioxide was used in this study and stock solutions were prepared. Arsenic trioxide LC_{50} values were 3.28 ppm respectively taken as sublethal concentration for this study. Twenty fish were selected and divided into 2 groups of 10 each and kept in 10 litre capacity tank. The first group was maintained in free from arsenic trioxide and served as the control. The second groups were exposed to arsenic trioxide for 28 days respectively. After this experimental period, the fish were sacrificed and gill tissues were removed from exposure period.

Spectroscopic analysis

FT-IR spectra were recorded at room temperature (25 ± 1C) in the 4000– 1000 cm^{-1} region with a Perkin Elmer-Spectrum RI Spectrometer equipped with a mullard I-alanine doped deuterated triglycine sulphate (DTGS) detector installed at Department of chemistry lab, Annamalai University. The instrument was under continuous dry nitrogen purge to eliminate atmospheric water vapour. Interferograms were averaged for 100 scans at 4 cm^{-1} resolution. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra automatically. The frequencies for all sharp bands were accurate to 0.001 cm^{-1}. Absorption intensity of the peaks was calculated with base-line method. The spectra were analyzed using origin 8.0 software.

To find out the number of peaks in the amide I region for the curve-fitting process, the resultant spectra were smoothed with 9-point Savitsky–Golay smooth function to remove the noise and the second derivative spectra were obtained using origin 8.0 software. After base-line correction and area normalized, the best bit for decomposing the amide I bands in the region between 1710 and 1590 cm^{-1} was obtained by Gaussian components using the same software. The curve-fitting is performed by step-wise iterative adjustment towards a minimum root-mean-square error of the different parameters determining the shape and position of the absorption peaks [5,9]. Band shape was considered Gaussian in all instances and base-line was always linear. The second derivative spectral analysis was applied to locate positions and assign them different functional groups of the overlapping components of the bands as performed by others[5,9]. The proportion of a component was computed to be the fractional area of the corresponding peak, divided by the sum of the areas of all the peaks. Thus, the areas corresponding to the different types of secondary structures are quantitatively and qualitatively evaluated by integration and curve fitting.

3. RESULTS

In the present study, the effect of arsenic treated on the biochemical content of fish gill tissues is investigated using FT-IR spectroscopy by monitoring different functional groups. (**Figure 1 & 2**) shows the representative FT-IR spectra obtained from the control and arsenic intoxicated fish gill tissues in the 4000–1000 cm^{-1} region. (**Table 1**)

It is based on the probable frequencies of vibrations of control gill and the arsenic exposed fish gill frequencies reported in the references and

their relative intensity in the FT-IR spectra from the figure, the FT-IR spectrum of the gill of *Ctenopharyngodon idella* is quite complex and contains several bands belonging to proteins, lipids, glycogen and nucleic acids.

Figure 1 shows the spectrum of the control gill is normalized with respect to the band ~3447 cm^{-1} is assigned as the N-H stretching of proteins. In figure 2, the N-H stretching bands of proteins band shift at ~3422 cm^{-1} (Amide A: N-H stretching band of proteins). It indicates the N-H stretching bands of proteins decreased in the gill of fish exposed to arsenic.

The band absorption at ~3003 cm^{-1} (Fig.1) is assigned CH_2 symmetric stretching of lipids is observed in the gill of the control fish.

The band absorption at ~2924 cm^{-1} (Fig.1) is assigned CH_3 asymmetric stretching: mainly lipids. In figure 2, the CH_3 asymmetric stretching of mainly lipids band is assigned at same ~2924 cm^{-1}.

The band absorption at ~2853 cm^{-1} (Fig.1) is assigned CH_2 symmetric stretching: mainly lipids. In figure 2, the CH_2 symmetric stretching: mainly lipids band is assigned at same ~2853 cm^{-1}.

The bands absorption at ~1745 cm^{-1} (Fig.1) is assigned as the C=0 stretching: mainly lipids. In figure 2, the band shifts at ~1742 cm^{-1} C=0 stretching: mainly lipids. It indicates the C=0 stretching bands of lipids decreased in the gill of fish exposed to arsenic.

The sharp band absorption at ~1656 cm^{-1} (Fig.2) is assigned as the Amide II: C=O stretching of proteins is observed in the gill of the fish exposed to arsenic.

The sharp bands absorption at ~1652 cm^{-1} (Fig.1) corresponds to amide II vibration of structural proteins, respectively. The amide II absorption is mainly associated with the C=O stretching vibration of the protein amide. The sharp bands observed at ~1638 cm^{-1} (Fig.2) the amide II absorption is mainly associated with the C=O stretching vibration of the protein amide is shifted due to exposed to arsenic.

The sharp bands absorption at ~1562 cm^{-1} (Fig 1) corresponds to amide II vibration of structural proteins, respectively. The amide II absorption is mainly associated with the N-H bending and C-N stretching vibration of the protein amide. The sharp bands observed at ~1545 $cm^{-1.}$ (Fig 2) the amide II absorption is mainly associated with the N-H bending and C-N stretching vibration of the protein amide is shifted due to exposed to arsenic.

The bands absorption at ~1458 cm^{-1} (Fig.1) is assigned as the N-H bending of mainly amino acids. In figure 2, the band shifts at ~1460 cm^{-1} is assigned as the N-H bending of mainly amino acids. It indicates the N-H bending of amino acids increased in the gill of fish exposed to arsenic.

Figure 1 and 2, the bands at ~1377 cm^{-1} and ~1395 cm^{-1} arise mainly from the CH_3 symmetric bending of lipid respectively. Figure 2, the bands at ~1235 cm^{-1} is assigned PO_2 asymmetric stretching: mainly phospholipids.

Figure 1 and 2, the bands at ~1164 cm^{-1} and ~1170 cm^{-1} is assigned as the C-O-C asymmetric stretching of glycogen is respectively. Figure 1 and 2, the bands at ~1116 cm^{-1} and ~1091 cm^{-1} is assigned C-O stretching of glycogen. It indicates the C-O stretching of glycogen increased in the gill of fish exposed to arsenic.

The bands absorption at ~970 cm^{-1} (Fig.2) is assigned as the $C\text{-}N^{+}\text{-}C$ symmetric stretching of nucleic acids is observed in the gill of the arsenic exposed fish. Figure 1 bands at ~723 cm^{-1} is assigned C-C bending of lipids are observed in the gill of control fish.

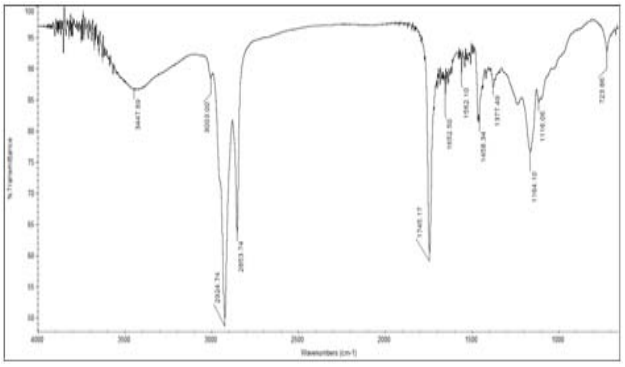

Figure 1: Representative FT-R spectra of the control gill tissue of freshwater fish, *Ctenopharyngodon idella* in the 4000-1000 cm^{-1} region

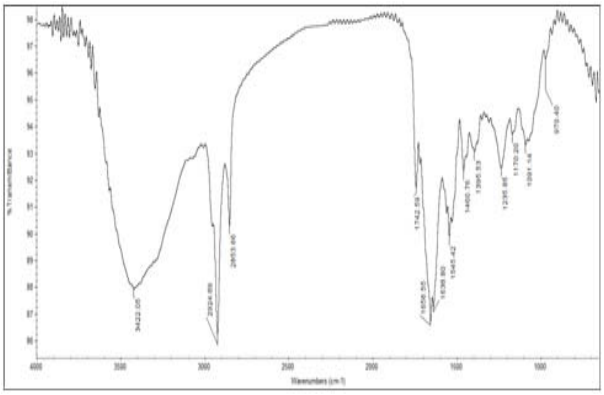

Figure 2: Representative FT-R spectra of the arsenic exposed gill tissue of freshwater fish, *Ctenopharyngodon idella* in the 4000-1000 cm^{-1} region

Table 1: General band assignments of the FT-IR spectra of the control and arsenic trioxide treated gill tissue of fish, *Ctenopharyngodon della*

Wave number in cm^{-1}			Vibrational assignment
Peak No	Control	Arsenic	
1	3447	3422	Amide A: mainly N–H stretching of proteins
2	3003	-	CH_2 symmetric stretching: mainly lipids
3	2924	2924	CH_3 asymmetric stretching: mainly lipids
4	2853	2853	CH_2 symmetric stretching: mainly lipids
5	1745	1742	C=O stretching: mainly lipids
6	-	1656	Amide II: C=O stretching of proteins
7	1652	1638	Amide II: C=O stretching of proteins
8	1562	1545	Amide II: N-H bending and C-N stretching of proteins
9	1458	1460	N-H bending: Amino acids
10	1377	1395	CH_3 symmetric bending: mainly lipids
11	-	1235	PO_2 asymmetric stretching: mainly phospholipids
12	1164	1170	C-O-C asymmetric stretching of glycogen
13	1116	1091	C-O stretching of glycogen
14	-	970	$C\text{-}N^{+}\text{-}C$ symmetric stretching of nucleic acids
15	723	-	C-C bending: mainly lipids

4. DISCUSSION

FT-IR spectroscopy is capable of providing strong insight into the structural and functional alterations induced by various factors due to it's high sensitivity [5,9]. The present study carried out to analyze the effect of arsenic treated on the biochemical content of gill, tissue of *Ctenopharyngodon idella* for 28 days exposure period. The result of the present study suggests that, the arsenic intoxication induces significant alteration on the major biochemical constituents such as proteins, lipids, carbohydrates and nucleic acids. According to [10] an increment or a decrement in the ratio of the intensities of the amide bands at 1540 cm^{-1} and 1650 cm^{-1} could be attributed to a changes in the composition of the whole protein pattern, the ratio of the peak intensities of the bands at 1080 and 1540 cm^{-1} was used by [11] to indicate the relative concentration of the glycoprotein content.

The significant alterations of present FTIR investigations, it has been observed that in the gill tissues, the amount of protein contents were found to be decreased whereas, the amount of lipid content were decreased and/or increased in the selected tissue region than the control fish, may be suggested due to the exposure of arsenic trioxide after 28 days. These changes suggested that, free radical damage could cause a reduction in protein synthesis [12]. Further, changes due to less proteolytic and more lipolytic activity. Similar results were observed in *Cyprinus carpio* when exposed to nickel [13], in *Labeo rohita* exposed to arsenic [14].

The decreasing intensity trend in *rainbow trout* gill treated with 17β estradiol with nonylphenol [4]. This decreased intensities of the amide I and II bands could be interpreted as the result of alteration of the protein synthesis and the protein structure due to arsenic intoxication. [15] Has reported that arsenic compounds interact with thiol groups strongly and specifically, interactions between trivalent arsenic and thiol containing residues in proteins and peptides have generally been regarded as the basis for the effects of this metalloid on the structure and function of these molecules [16]. Loss of thiol groups is considered to be one of the immediate responses to an elevation in the level of oxidation stress [17].

An important factor affecting the membrane structure and dynamics is the amount of proteins and lipids in the membranes [18]. [19] Have also reported decreased sulfhydryl proteins in the rat brain regions due to arsenic treatment. [20] Have noticed a fall in the level of total protein content in the gill and liver tissues of *Channa punctatus* exposed to mercury. [21] Have reported that the decreased quantity of protein in the selected tissues might be due to its conversion to amino acid residue in order to increase amino acid pool. The decreased protein content in the fish tissue was due to non-selective blocking of phosphorylation process in the gill tissue and also it can be influenced by large number of exogeneous substances, mainly through a reduction of protein synthesis capacity of the endoplasmic reticulum in the cells. [22] have reported a decrement level of total protein content

in the gill tissues of *Labeo rohita* exposed to arsenic. Decreased levels of protein observed in the arsenic intoxicated gill tissues of *Labeo rohita* may be due to the binding of arsenic with various sulfhydryls that exist in the cell, as suggested by [19]. [23] Have also observed a reduction in lipid content which could be due to the reduced synthesis of lipid or increased activity of lipase involved in oxidation of lipid. Decrease in the ratio suggests a decrease in the protein content [24,25].

The shifting in the frequency of CH_3 asymmetric stretching band to lower values revealed that, the librational freedom of the acyl chains of the phospholipids decreased in the central area of the bilayer of arsenic intoxicated rat liver microsomal membranes. The hydrogen bonding might be between water molecules and the oxygen molecules of phosphate groups of phospholipids. This may create a difference in packing of phospholipid molecules [4].

Several investigators have studied in fish tissues by using Fourier Transform Infrared spectroscopy (FT-IR) by [5,9,19,27,25,26,28]. Structural, compositional and functional changes in *rainbow trout* by using Fourier Transform Infrared spectroscopy reported by [29].

CONCLUSION

In conclusion, the present investigation shows that, the gill tissues are vulnerable to arsenic intoxication. The result further suggests that arsenic intoxication induces significant alteration on the major biochemical constituents such as proteins, lipids and nucleic acids, which can be easily evidenced by FT-IR spectroscopy.

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