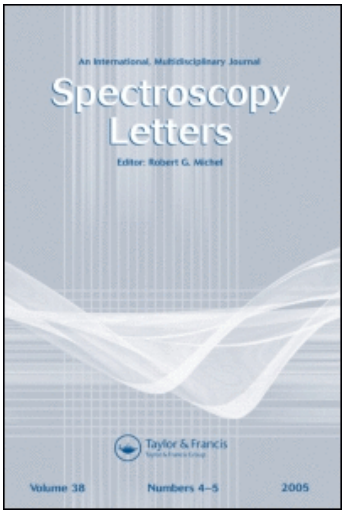

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Arsenic-Induced Biochemical Changes in *Labeo rohita* Kidney: An FTIR Study

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ABSTRACT Arsenic is a toxic heavy metal that occurs naturally in water, soil, and air. It is widespread in the environment as a consequence of both anthropogenic and natural processes. In the current study, an attempt has been made to analyze the arsenic-induced molecular changes in macromolecular components like proteins and lipids in the kidney tissues of edible fish *Labeo rohita* using Fourier transform infrared (FTIR) spectroscopy. The FTIR spectrum of kidney tissue is quite complex and contains several bands arising from the contribution of different functional groups. The detailed spectral analyses were performed in three distinct wave number regions, namely 3600–3050 cm^{-1}, 3050–2800 cm^{-1}, and 1800–800 cm^{-1}. The current study shows that the kidney tissues are more vulnerable to arsenic intoxication. FTIR spectra reveal significant differences in both absorbance intensities and areas between control and arsenic-intoxicated kidney tissues; this result indicates that arsenic intoxication induces significant alteration on the major biochemical constituents such as lipids and proteins and leads to compositional and structural changes in kidney tissues at the molecular level. The current study confirms that FTIR spectroscopy can be successfully applied to toxicologic and biological studies.

KEYWORDS *Labeo rohita*, arsenic, FTIR, kidney, lipids, proteins

INTRODUCTION

Heavy metal release into the environment has alarmingly increased during recent years because of emission from automobiles, coal burning, mining, industrial activities, and trash incineration. Most heavy metals released into the environment find their way into the aquatic system as a result of direct input, atmospheric deposition, and erosion caused by rain. As a result, aquatic animals are exposed to elevated levels of heavy metals. Arsenic is one such heavy metal that occurs naturally in water, soil, and air. It is widespread in the environment as a consequence of both anthropogenic and natural processes. Inorganic as well as organic forms of arsenic are present in the environment, and the former seems to be more toxic and slightly more accumulated in some freshwater aquatic species than is the latter.[1] Trivalent arsenic is considered more toxic to aquatic biota than is the inorganic pentavalent form.

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Fourier transform infrared spectroscopy is used to measure the vibrational modes of functional groups of molecules and is sensitive to molecular structure, conformation, and environment.[2,3] In recent years, efforts have been made to apply this technique to quantify those biochemical components such as proteins and lipids in biological samples.[2,3] Fish have been widely used as models for monitoring toxic chemicals in aquatic environments. Arsenic is actively metabolized in the tissues of fish especially in organs such as liver and kidney.[4] In the current study, an attempt has been made to analyze the arsenic-induced molecular changes in macromolecular components like proteins and lipids in kidney tissues of edible fish *Labeo rohita* using FTIR spectroscopy. In the current study, rohu (*Labeo rohita*) is used because it is a traditional nutritious food fish for people in India. Further, it is available in abundance and withstands a wide range of experimental conditions.

MATERIALS AND METHODS

Test Species

Freshwater fingerlings of *Labeo rohita* of length 6 ± 1 cm and weight 8 ± 1 g were procured from the fish farm at Puthur, Tamil Nadu, India. The collected fish were acclimated to laboratory conditions in dechlorinated tap water for 15 days.[5] The fish were fed with commercial fish feed (The Himalaya Company, Bangalore, India) during acclimation.

Test Chemicals

The AnalaR grade Arsenic trioxide (As_2O_3) obtained from Sigma Aldrich Company (Bangalore, India) was used without further purification.

Lethality Studies

The LC_{50} value for arsenic was determined by using the method of Litchfield and Wilcoxon[6] and was found to be 124.5 ppm. Arsenic stock solution was prepared by dissolving 1.3202 g of As_2O_3 in 1 L of dilute acidic water. The acclimated fish were stocked in 30-L glass troughs of dimension $60 \times 30 \times 30$ cm equipped with a continuous air supply. The physicochemical parameters such as pH, total alkalinity, total hardness, calcium, and magnesium were measured according to APHA[7] and maintained throughout the experiment (7.5, 120 mg/L, 200 mg/L, 50 mg/L, and 18 mg/L). The water was changed along with waste feed and fecal materials periodically by slowly siphoning the water from each container. The containers were refilled and redosed with metal toxicant daily.

Experimental Study

The experimental design and calculations for the acute toxicity were based on the procedure given by Sparks.[8] The acclimated test fish were divided into two groups, each containing 25 fish. Group I was used as control and reared in metal-free water for 14 days. The test fish belonging to group II were exposed to higher sublethal concentration (41.5 ppm) of arsenic for 14 days (subacute exposure). After this period, the fish were sacrificed and kidney tissues removed and stored at $-80°C$ until sample preparation for FTIR spectroscopic studies.[9]

Sample Preparation

The kidney tissues were first kept in a drier overnight to remove the water content from the samples. The dried samples were ground with an agate mortar and pestle. The powdered samples were thoroughly mixed with completely dried potassium bromide at a ratio of 1 mg sample to 100 mg KBr. Then, the samples were pressed into pellets in an evacuated die to produce a clear transparent disk of 13 mm diameter and 1 mm thickness.

Spectroscopic Analysis

FTIR spectra were recorded in the 4000–400 cm^{-1} spectral region at room temperature ($25 \pm 1°C$) on a Perkin Elmer–Spectrum RxI Spectrometer (PerkinElmer, USA) equipped with a mullard I–alanine doped triglycine sulfate (DTGS) detector installed at the Centralised Instrumentation and Services Laboratory, Annamalai University. For each spectrum, 100 scans were co-added at a spectral resolution of ± 4 cm^{-1}. The spectrometer was continuously purged with dry nitrogen. The frequencies for all sharp bands were accurate to 0.001 cm^{-1}. Each sample was scanned under the same conditions with three different pellets. These replicates were averaged and then used. Absorption intensity of the peaks

was calculated by the base-line method. Special care was taken to prepare the pellets at the same thickness by taking the same amount of sample and applying the same pressure. Therefore, in the current study it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups.[10] The spectra were analyzed using Origin 8.0 software (OriginLab Corporation, Massachusett, USA).

RESULTS AND DISCUSSION

The current study was carried out to analyze the effect of arsenic intoxication on the biochemical contents of the kidney tissues of *Labeo rohita* fingerlings using FTIR spectroscopy. The intensity and/or more accurately the area of the absorption bands in the FTIR spectrum is directly related to the concentration of the molecules.[10] Figure 1 shows the representative FTIR spectrum obtained from untreated (control) fish kidney tissues in the 4000–400 cm^{-1} region. The main absorption bands are defined in detail in Table 1. As seen from Fig. 1, the spectrum of kidney tissues is quite complex and contains several bands arising from the contribution of different functional groups belonging to lipids, proteins, and others. Therefore, the detailed spectral analyses were performed in three distinct wave number ranges, namely 3600–3050 cm^{-1}, 3050–2800 cm^{-1}, and 1800–800 cm^{-1}.[9–11] For the accurate measurements of the spectral parameters, all the spectra were normalized with respect to specific selected bands and considered separately. The band area values of the selected bands are presented in Table 2.

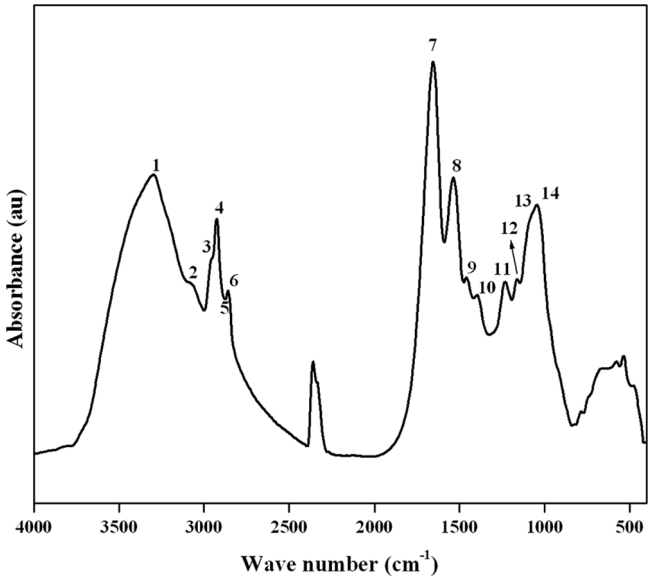

FIGURE 1 The representative FTIR spectrum of the control kidney tissues of *Labeo rohita* in the 4000–800 cm^{-1} region.

TABLE 1 General Band Assignments of the FTIR Spectrum of Control Kidney Tissues of *Labeo rohita* Based on Literature

Peak no.	Wave number in cm^{-1}	Definition of the spectral assignments
1	3294(s)	Amide A: mainly N–H stretching of proteins
2	3078(w)	Amide B: N–H stretching of proteins
3	2957(s)	CH_3 asymmetric stretch: mainly lipids
4	2925(s)	CH_2 asymmetric stretch: mainly lipids
5	2872 (w)	CH_3 symmetric stretch: mainly proteins
6	2853 (s)	CH_2 symmetric stretch: mainly lipids
7	1655 (s)	Amide I: C=O stretching of proteins
8	1542 (s)	Amide II: N–H bending and C–N stretching of proteins
9	1458 (m)	CH_2 bending: mainly lipids
10	1399 (m)	COO^- symmetric stretch: fatty acids and amino acids
11	1233 (m)	PO_2^- asymmetric stretch: mainly phospholipids
12	1160 (m)	C–O asymmetric stretching of glycogen
13	1081 (s)	PO_2^- symmetric stretch: mainly nucleic acids
14	1060 (s)	C–O stretching: polysaccharides

s, strong; m, medium; w, weak.

Figure 2 shows the FTIR spectra of control and arsenic-intoxicated kidney tissues in the 3600–3050 cm^{-1} region. The spectra were normalized with respect to the 2925 cm^{-1} band. The band observed at

TABLE 2 The Band Area Values of Selected Bands for Control and Arsenic Intoxicated Kidney Tissues

Wave number (cm^{-1})	Control	Arsenic Intoxicated
3294	129.71 ± 2.154	$124.73 \pm 1.582\downarrow$
3078	0.41 ± 0.034	$0.23 \pm 0.012\downarrow$
2957	1.55 ± 0.051	$1.17 \pm 0.019\downarrow$
2925	5.03 ± 0.084	$5.17 \pm 0.062\uparrow$
2872	0.09 ± 0.005	$0.05 \pm 0.003\downarrow$
2853	1.16 ± 0.009	$1.46 \pm 0.013\uparrow$
1655	61.20 ± 1.881	$56.34 \pm 1.284\downarrow$
1542	13.86 ± 0.458	$13.43 \pm 0.248\downarrow$
1458	1.70 ± 0.551	$0.82 \pm 0.345\downarrow$
1399	4.28 ± 0.323	$2.48 \pm 0.220\downarrow$
1233	5.94 ± 0.026	$4.89 \pm 0.047\downarrow$
1081	18.22 ± 1.080	$13.46 \pm 1.044\downarrow$

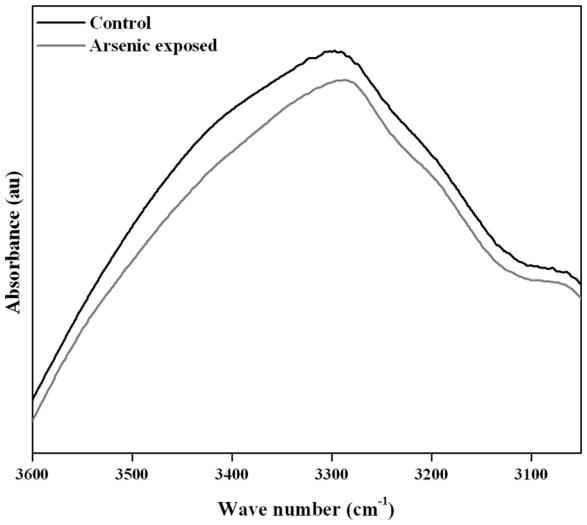

FIGURE 2 FTIR spectra of the control and arsenic intoxicated kidney tissues in the 3600–3050 cm^{-1} region. The spectra were normalized with respect to the band at 2925 cm^{-1}.

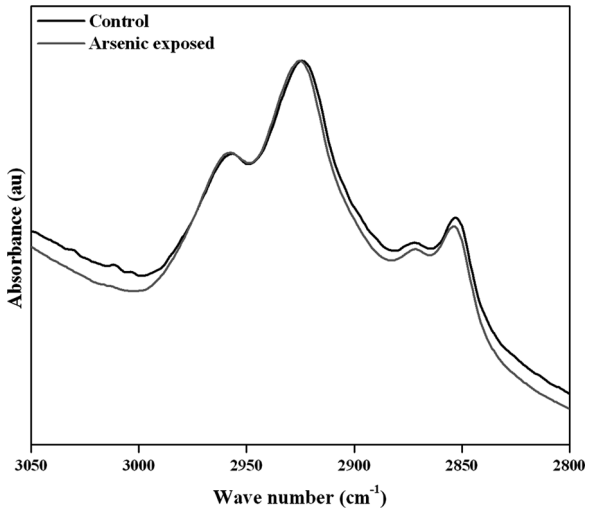

FIGURE 3 FTIR spectra of the control arsenic intoxicated kidney tissues in the 3050–2800 cm^{-1} region. The spectra were normalized with respect to the band at 2925 cm^{-1}.

3294 cm^{-1} corresponds with the amide A stretching mode that can generally be associated with N–H groups. The band at 3078 cm^{-1} is due to N–H stretching proteins of amide B. The numerical calculations reveal that arsenic intoxication leads to decrease in the peak area of the amide A and B bands respectively from 129.71 ± 2.154 and 0.41 ± 0.034 in the control group to 124.73 ± 1.582 and 0.23 ± 0.012 in the arsenic-intoxicated group.

The significant decrease in the peak area of the amide A band set off by arsenic means that it led to a decrease in the protein quantity of the system. This could be a supportive sign of the destructive effect of arsenic, as it was suggested[12] that free radical damage could cause a reduction in protein synthesis. Further, such an oxidative action of arsenic is supported by the observation that arsenic treatment decreases the protein content in the brain tissues of rat.[13]

Figure 3 shows the FTIR spectra of control and arsenic-intoxicated kidney tissues in the 3050–2800 cm^{-1} region. The spectra were normalized with respect to the CH_2 antisymmetric stretching mode (2925 cm^{-1}). As can be seen from Fig. 3, pronounced differences were seen in this region where the spectrum is populated by absorptions arising from the C–H stretching vibrations of $–CH_2$ and $–CH_3$ groups. The band area of the CH_3 asymmetric stretching band observed at 2957 cm^{-1} is decreased. The CH_2 asymmetric stretching band observed at 2925 cm^{-1} in the control tissues has been shifted (2924 cm^{-1}) in the arsenic-intoxicated tissues. The CH_3 symmetric stretching band observed at 2872 cm^{-1} diminished in the arsenic-intoxicated kidney spectrum. Moreover, the area of the CH_2 symmetric stretching vibration band at 2853 cm^{-1} significantly increased in the arsenic-intoxicated kidney tissues compared wih that of control.

Lipids play a key role in membrane fluidity. By affecting the conformation of membrane proteins, they govern exposure and diffusion of membrane components. The changes in lipid fluidity can be detected by analyzing the methylene stretching bands of the lipid hydrocarbon chains. From Table 2, the band area of the CH_3 asymmetric stretching vibration (2957 cm^{-1}) decreases from 1.55 ± 0.051 to 1.17 ± 0.019 as a consequence of arsenic intoxication. This decrease indicates a change in the composition of the acyl chains.[10] In addition, the area of the symmetric CH_2 stretching band was found to be significantly increased in the arsenic-intoxicated tissues. This result suggests an increased proportion of the CH_2 groups in the arsenic-intoxicated tissues.

Figure 4 shows the FTIR spectra of control and arsenic-intoxicated kidney tissues in the 1800–800 cm^{-1} region. The spectra were normalized with respect to the amide I band at 1655 cm^{-1}. As seen from the figure, the absorbance of the bands were generally decreased in the arsenic-intoxicated

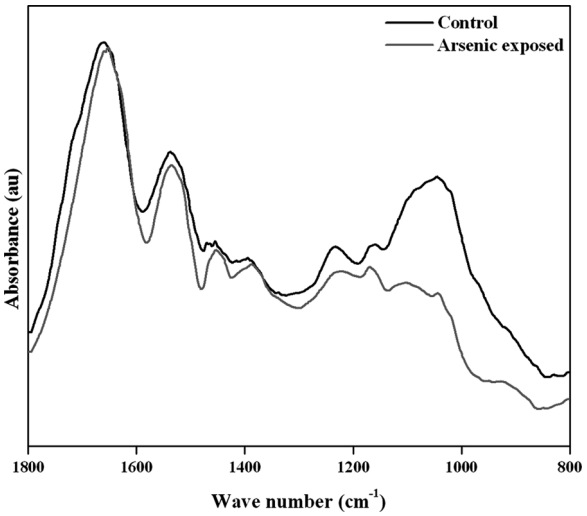

FIGURE 4 **FTIR spectra of the control arsenic intoxicated kidney tissues in the 1800–800 cm^{-1} region. The spectra were normalized with respect to the band at 1655 cm^{-1}.**

tissues. The sharp bands at 1655 cm^{-1} and 1542 cm^{-1} correspond with amide I and amide II vibrations of structural proteins respectively.[2,3,9,10] The amide I absorption is mainly associated with the protein amide in-plane C=O stretching vibrations. The position of this absorption is sensitive to protein conformations. The amide II absorption arises from N–H bending vibration (60%) coupled with C–N stretching (40%) mode of the polypeptide and protein backbone. Both of them are conformationally sensitive and thus are often used to determine protein secondary structures.[2,3,14] The amide I and II peaks observed at 1655 cm^{-1} and 1542 cm^{-1} suggest that the protein is dominated by α-helical structure in the kidney tissues. It is seen from Table 2 that the area of amide I and amide II bands decreases respectively from 61.20 ± 1.881 to 56.34 ± 1.284 and 13.86 ± 0.458 to 13.43 ± 0.248 in the arsenic-intoxicated tissues. These changes reflect the loss of protein levels in the arsenic-intoxicated tissues. This loss of protein provides verification of increased protein oxidation in the kidney tissues with arsenic exposure. Loss of function of protein may result from a change in critical side chain or from a break in the hydrogen or disulfide bonds, which maintain the secondary and tertiary structures. This break can lead to a partial unfolding of the tightly coiled peptide chain. It results in a disorganization of the internal structure.[15] Samuel et al.[13] have also reported decreased sulfhydryl proteins in rat-brain regions due to arsenic treatment. Decreased levels of protein thiols observed in the current study in the arsenic-intoxicated gill tissues of fish are suggestive of an excess free radical production and binding of arsenic with various sulfhydryls that exist in the tissue cells. Webb[16] 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.[17] Loss of thiol groups is considered as one of the immediate responses to an elevation in the level of oxidation stress.[18]

The band observed at 1458 cm^{-1} is due to bending vibration of the CH_2 in the lipids and proteins. As seen from Table 2, a decrease in the area (52%) was observed at 1458 cm^{-1}. The band centered at 1399 cm^{-1} can be attributed to COO^- symmetric stretching vibration of amino acid side chains and fatty acids. A decrease of 42% was also observed in the area of this band. The bands at 1233 cm^{-1} and 1081 cm^{-1} are mainly due to PO_2^- asymmetric and symmetric stretching of phospholipids, respectively. The peak height and area values of both phosphate asymmetric and symmetric stretching band were also decreased in the arsenic-intoxicated tissues.

CONCLUSIONS

In conclusion, the current study shows that the kidney tissues are more vulnerable to arsenic intoxication. The result further indicates that arsenic intoxication induces significant alteration on the major biochemical constituents such as lipids and proteins and leads to compositional and structural changes in kidney tissues at molecular level. The current work confirms that FTIR spectroscopy can be successfully applied to toxicological and biological studies.

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