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Original Article

EFFICACY OF MICRODOSES POTENTIZED HOMEOPATHIC DRUG, ARSENICUM ALBUM INDUCED BY ARSENIC TRIOXIDE IN ZEBRAFISH (DANIO RERIO)-A FTIR STUDY IN GILLS

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ABSTRACT

Objective: Arsenic, one of the major natural as well as anthropogenic pollutant produces biochemical changes in the organs of animals due to its potential toxicity. Arsenic is toxic to fish and may be taken through gills and skin. In the current work, arsenic trioxide-treated fishes were prescribed with potentized homoeopathic drug, ars alb 30C, and ars alb 200C. The biochemical contents in exposed gill tissues of zebra fish (*Danio rerio*) were analysed using Fourier transform infrared spectroscopy (FTIR).

Methods: The acclimatised fish were treated with arsenic trioxide and LC_{50} was calculated. Along with arsenic trioxide, the homoeopathic drug ars alb 30C and ars alb 200C were given three drops per litre. After a period of 7 and 14 d, the gills of exposed fish were dissected. The samples were lyophilized and analysed in FTIR.

Results: In arsenic exposed fish, the amine N-H stretch was different when compared to the control and the treated different potencies of ars alb 30C and 200C respectively at 7days. Whereas at 14 d the arsenic exposed fish the amine N-H stretch was drastically reduced. Notably, in treating different potencies of ars alb 30C and 200C, the amine N-H stretch was increased.

Conclusion: It was found that the higher the potency that is ars alb 200C was more effective against Arsenic toxicity. It shows higher dilution may be efficacious.

Keywords: Arsenic trioxide, Ars alb, Gills, FTIR

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INTRODUCTION

Arsenic one of the major contaminant in groundwater, possibly cause many sufferings in humans as well as organisms in water [1]. In West Bengal, India, the amount of arsenic, was getting discharged progressively, crumbled in water, with aquatic and soil sediment [2]. Absorption of arsenic is being controlled by a mixture of input and removal mechanisms happened in the aquatic environment. Accumulation of arsenic in the physical system leads to physiological and biochemical disorders. An aquatic ecosystem is a disparate association of organisms and biotic factors, which are interrelated, even the micro-modifications in the environment can change the nature and conduct of the organisms. Freshwater fish constitute one of the major origins of cheap nutrition for human beings. Biochemical composition of proteins, carbohydrates, free amino acids, lipids and mineral contents are the important nutritional values of fish [3]. The global yield of farmed fish more than doubled in value over the past decade. The fish developed from farming activities currently account for over one after parting of all fish directly consumed by humans. As the human population continues to rise, the reliance on farmed fish production as an important source of protein will also increase [4]. The consequence of inorganic arsenic species might result in a larger number of adverse health effects to the cosmopolitan public. Thus, it is important to evaluate the inorganic arsenic levels of farmed seafood in arsenicpolluted fields [5]. These answers indicate that the dwellers of the arsenic polluted region are being subjected to moderately elevated arsenic exposure through the use of tilapia and shrimp raised in aquaculture ponds.

The bulk of toxic stuff initiates biochemical alterations like inhibition of the enzyme system, the amendment in the level of enzyme and individual alteration in the permeability of biological membranes [6]. The rate of elimination of arsenic became more composite because of the poor people health and hygiene status and the relatively low affordability of the greater majority of the population surviving in contaminated zones. And for the lack of awareness of

the potential effects of arsenic poisoning in most of them. Mainly with such an environment, the initial object was to find out suitable antagonists of arsenic poisoning, which should be easily executable, in force in less quantity and low price.

Earlier certain studies were getting on with mice a potentized homoeopathic drug, ars alb 30C and it presents a promising result [7]. The biomedical technique Fourier Transform Infrared Spectroscopy (FTIR) is unitary of the techniques that can potentially discover a wealth of qualitative and quantitative information about a given biological sample. Tissue components, including membranes, proteins, nucleic acids and polysaccharide compounds can be evaluated by FTIR spectroscopy. This indicates that this technique is valuable for analysing complicated biological materials, including tissues, body fluids or cell cultures. The transformation in the top positions, bandwidth and the saturation of the stripes, all give valuable structural and operational information which may have diagnostic value for biological systems. Thus, it seemed exciting to apply FTIR spectroscopy to monitor disease induced and arsenic toxicity induced biochemical changes occurring in any living organism and to evaluate the quantitative and semi-quantitative effects of various factors on biological structures.

MATERIALS AND METHODS

Test species

Zebrafish (*D. rerio*) adults (length: 4 ± 0.2 ; weight: 2 ± 0.3) total of 100 was obtained from Muthupandi fish farm, Kadachanenthal, Madurai District, Tamil Nadu, India. It was acclimatised in laboratory conditions in glass tanks for a minimal period of 14 d. It was maintained under temperature conditions of 25 ± 2 °C, 12 h/12 h light/dark cycle. At the time of the experiment, the fish were fed with artemia.

Test chemicals

Arsenic trioxide was purchased from Karnataka fine chemicals and arsenicum album was purchased from Dr Reckweg, Germany.

Lethality studies

The LC₅₀ values for arsenic trioxide were determined by using the arithmetic method of Sprague [8] and were set up to be $400\mu M$. Fishes were maintained in a glass tank of 20L capacity along with continuous air supply throughout the experiment. The physicochemical characteristics of test water were maintained accordingly by APHA [9] standards, such as pH (7–7.2), total alkalinity (125–131mEq/l), total hardness (200–205 mg/l as CaCO₃), calcium (50–56 mg/l), magnesium (18–22 mg/l) and DO (89–90 mg/l). During the experimental period, the water was changed every day and the fecal materials were removed. Throughout the experiment, physicochemical parameters were determined.

Experimental study

The observational work was conducted to examine the effect of homoeopathic drug ars alb by the method of Sparks [10]. The fish were acclimatized and grouped as follows in triplicate containing 10 fishes in each group and it was divided into Group I–VII such as,

Group I: positive control which is As_2O_3 intoxicated group, Group II: As_2O_3 intoxicated ars alb 200C group, Group III: As_2O_3 intoxicated ars alb 30C group, Group IV: act as alcohol control since ethyl alcohol was considered as the vehicle of the homeopathic remedies, Group V: fish treated with ars alb 200C alone, Group VI: fish treated with ars alb 30C alone and Group VII: negative Control, it consists of healthy fish not treated with arsenic.

Three drops per litre of ars alb 30C and ars alb 200C were given thrice a day to As_2O_3 intoxicated Group. The fishes were exposed to arsenic trioxide for 14 d (acute). After the experimental period was over, the gill tissues of the exposed fishes were removed for further process.

Sample preparation

The collected gill tissues were dried by lyophilizer (Martin Christ, Deutschland) for 12 h. After drying the samples were ground to obtain the powder form of the gill, following which it was mixed in a ratio of 1:100 with dried potassium bromide and 5 tons of pressure was given for 5 min to make a transparent KBr disc.

Spectroscopic measurement

The FTIR spectroscopic measurement was done using Spectrum Two™ from PerkinElmer. Wavelength ranging from 4000 to 400 cm¹was covered by this FTIR spectrometer. The intensity peak absorbed was calculated with ORIGIN 8.0 software.

RESULTS

The present study deals with the effect of ars alb intoxicated with arsenic and the changes in biochemical contents of $\it D. rerio$ gills using FTIR technique. The average FTIR Spectra obtained from control, and the other groups specifically. As_2O_3 intoxicated, As_2O_3 intoxicated ars alb 200C, As_2O_3 intoxicated ars alb 30C, ethyl alcohol, ars alb 200C and ars alb 30C. Obtained peak region and spectra are represented in fig. 1 and 2.

The structural, as well as functional information value, were conveniently obtained by the shifts in peak position [11]. Important functional groups such as proteins, lipids, ester, amide and nucleic acids can be validated via FTIR.

The spectrum consists of several bands arising from the functional groups belonging to the vibrational assignments of FTIR spectra for control, As_2O_3 Intoxicated, As_2O_3 intoxicated ars alb 200C, As_2O_3 intoxicated ars alb 200C and ars alb 30C fish tissue samples in the 4000-400 cm $^{-1}$ for 7 d and 14 d respectively were presented in table 1 and table 2.

In fig. 1 the spectrum of control shows the band of $\sim 3401~cm^{-1}$ which has the vibrational assignment of an amine N-H stretch. 1g which is arsenic intoxicated showed decreased N-H stretching bands $\sim 3287~cm^{-1}$ of proteins, whereas in the case of 2g As₂O₃+ars alb 200C the rate of peak shift $\sim 3293~cm^{-1}$ was slightly varied in comparing to arsenic intoxicated. In the case of 3g As₂O₃+ars alb 30C, the rate of peak shift was similar to arsenic intoxicated since it contains low

dilution of arsenic trioxide. Its shows at $7\ d$ of treatment there is an effective change in vibrational assignment.

The absorptions in this area are dominated by the amide A of proteins and are due to stretching vibrations of N–H groups in the peptide chains of the protein macromolecules and intermolecular H-bonding. The position of amide A band in the spectra indicates that the N–H groups are associated because a maximum of the absorption bands of free N–H vibrations in protein macromolecules are located at $\sim 3480-3450$ cm⁻¹.

In fig. 2 the spectrum of control shows the band of $\sim 3401~\rm cm^{-1}$ which has the vibrational assignment of an amine N-H stretch. 2a1g which is arsenic intoxicated showed more decreased N-H stretching bands $\sim 3277~\rm cm^{-1}$ of proteins, whereas in the case of 2a2g As₂O₃+ars alb 200C the rate of peak shift $\sim 3304~\rm cm^{-1}$ was slightly varied in compared to arsenic intoxicated. In the case of 2a3g As₂O₃+ars alb 30C the rate of peak shift $\sim 3282~\rm cm^{-1}$ was similar to arsenic intoxicated since it contains low dilution of arsenic trioxide. Its shows at 7 d of treatment there is an effectual change in vibrational assignment.

In fig. 1 the spectrum of control shows the band of ${\sim}2922~cm^{\text{-}1} and {\sim}2853~cm^{\text{-}1} which have the vibrational assignment of an alkyl C-H stretch. 1g which is arsenic intoxicated showed decreased CH3 asymmetric stretching: mainly lipids bands <math display="inline">{\sim}2920~cm^{\text{-}1} whereas$ in the case of 2g As₂O₃+ars alb 200C and 3g As₂O₃+ars alb 30C the rate of peak shift ${\sim}2923~cm^{\text{-}1} remains$ same.

In fig. 2 the spectrum of control shows the band of $\sim\!2922~cm^{\text{-}1}\!and$ $\sim\!2853~cm^{\text{-}1}\!which$ have the vibrational assignment of an alkyl C-H stretch.

2a1g which is arsenic intoxicated showed decreased CH₃ asymmetric stretching: mainly lipids bands $\sim\!2920~cm^{\text{-1}}\text{whereas}$ in the case of 2a2g As₂O₃₊ars alb 200C CH₂ symmetric stretching: mainly lipids showed no peak value and 2a3g As₂O₃₊ars alb 30C the rate of peak shift $\sim\!2851~cm^{\text{-1}}\text{remains}$ closer to the central peak.

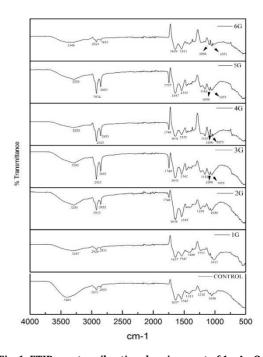


Fig. 1: FTIR spectra vibrational assignment of $1g-As_2O_3$ intoxicated, $2g-As_2O_3$ intoxicated ars alb 200C, $3g-As_2O_3$ intoxicated ars alb 30C, 4g-ethyl alcohol, 5g-ars alb 200C and 6g-ars alb 30C treated gills of D. rerio

In fig. 1 the spectrum of control shows the band of $\sim\!1637$ cm $^{\!-1}\!$ which has the vibrational assignment of amide C=0 Stretch.

1g which is arsenic intoxicated showed the same band as a control but later at the 14 d; the band was stretched $\sim 1647~cm^{-1}(fig.~2)$ of

amide II: C=0 stretching of proteins. whereas in the case of 2g As₂O₃+ars alb 200C the rate of peak shift increased to the wavelength of ~1646 cm⁻¹(fig. 1) later on the band was similar to

control $\sim 1638~cm^{-1}(fig.~2)$. In 3g As₂O₃+ars alb 30C the rate of peak shift was in increased condition arsenic intoxicated since it contains low dilution of arsenic trioxide.

Table 1: FTIR spectra vibrational assignment of 1g-As₂O₃ intoxicated, 2g-As₂O₃ intoxicated ars alb 200C, 3g-As₂O₃ intoxicated ars alb 30C, 4g-ethyl alcohol, 5g-ars alb 200C and 6g-ars alb 30C treated gills of *D. rerio*

S. No.	Peak posi	tion				Vibrational assignments		
	Control	2a1g	2a2g	2a3g	2a4g	2a5g	2a6g	-
1	3401	3277	3304	3282	3287	3299	3299	amine N-H stretch
2	2922	2924	2915	2922	2924	2924	2914	CH ₃ asymmetric stretching: mainly lipids
3	2853	-	-	2851	2853	2853	-	CH ₂ symmetric stretching: mainly lipids
4	1637	1647	1638	1650	1644	1646	1644	amide II: C=O stretching of proteins
5	1542	1539	1542	1547	1539	1545	1549	amide II: N-H bending and C-N stretching of proteins
6	1414	1410	1408	1410	1410	1404	1407	N-H bending: amino acids
7	1236	1233	1231	1234	1234	1234	1231	PO ₂ asymmetric stretching: mainly phospholipids
8	1050	1056	1049	1051	1088	1057	1047	C–C stretching of lipids

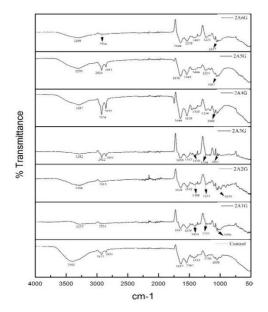


Fig. 2: FTIR spectra vibrational assignment of control 2a1g-As₂O₃ intoxicated, 2a2g-As₂O₃ intoxicated ars alb 200C, 2a3g-As₂O₃ intoxicated ars alb 30C, 2a4g-ethyl alcohol, 2a5g-ars alb 200C and 2a6g-ars alb 30C treated gills of *D. rerio*

In fig. 1 the spectrum of control shows the band of $\sim 1542~\rm cm^{-1}$ which has the vibrational assignment of amide II: N-H bending and C-N stretching of proteins. 1g which is arsenic intoxicated showed same band as control, but later at 14 d the band was decreased to $\sim 1539~\rm cm^{-1}(fig.\ 2)$ of proteins, whereas in case of 2g As₂O₃+ars alb 200C the rate of peak shift increased to $\sim 1545~\rm cm^{-1}$ (fig. 1) later on the band

was similar to control ~ 1542 cm⁻¹(fig. 2). In 3g As₂O₃+ars alb 30C showed the same band as a control, but later at 14 d the band was increased to ~ 1547 cm⁻¹(fig. 2) of proteins.

In fig. 1 the spectrum of control shows the band of $\sim\!1414~cm^{\!-\!1}$ which has the vibrational assignment of N-H bending: amino acids. 1g which is arsenic intoxicated showed decreased band of $\sim\!1408~cm^{\!-\!1}$, but later at 14 d the band was increased to wavelength $\sim\!1410~cm^{\!-\!1}$ (fig. 2), whereas in case of 2g As_2O_3+ars alb 200C the rate of peak shift decreased to $\sim\!1545~cm^{\!-\!1}$ (fig. 1) later on the band was increased to $\sim\!1408~cm^{\!-\!1}$ (fig. 2). In 3g As_2O_3+ars alb 30C showed no stretched band at 7 d and but later at 14 d the band was increased to $\sim\!1410~cm^{\!-\!1}$ (fig. 2).

In fig. 1 the spectrum of control shows the band of ~ 1236 cm⁻¹which has the vibrational assignment of PO₂ asymmetric stretching: mainly phospholipids.

1g which is arsenic intoxicated showed decreased band of $\sim\!1232$ cm $^{\!-1}$ on 7 d, but at 14 d the band was $\sim\!1233$ cm $^{\!-1}$ (fig. 2), whereas in case of 2g As₂O₃+ars alb 200C the rate of peak shift decreased to $\sim\!1236$ cm $^{\!-1}$ (fig. 1) later on the band was decreased to $\sim\!1231$ cm $^{\!-1}$ (fig. 2). In 3g As₂O₃+ars alb 30C showed stretched band was found to be $\sim\!1235$ cm $^{\!-1}$ at 7 d and but later at 14 d the band as reduced to $\sim\!1234$ cm $^{\!-1}$ (fig. 2).

No stretching band was available at ethyl alcohol and homoeopathic medicine ars alb 30C alone on the 7 d and the stretching peaks were observed at 14 d found to be decreased.

In fig. 1 the spectrum of control shows the band of ~ 1050 cm $^{-1}$ which have the vibrational assignment of C–C stretching of lipids.

1g which is arsenic intoxicated showed decreased band of $\sim\!1013$ cm $^1\!on$ 7 d but, at 14 d the band was $\sim\!1056$ cm $^1\!.$ Whereas in the case of 2g As₂O₃₊ars alb 200C the rate of peak shift was $\sim\!1058$ cm $^1\!$ (fig. 1) later on the band was decreased to $\sim\!1049$ cm $^1\!$ (fig. 2). In 3g As₂O₃₊ars alb 30C showed stretched band was found to be $\sim\!1055$ cm $^1\!$ at 7 d and but later at 14 d the band was reduced to $\sim\!1051$ cm $^1\!$ (fig. 2).

Table 2: FTIR spectra vibrational assignment of 2a1g-As₂O₃ intoxicated, 2a2g-As₂O₃ intoxicated ars alb 200C, 2a3g-As₂O₃ intoxicated ars alb 30C, 2a4g-ethyl alcohol, 2a5g-ars alb 200C and 2a6g-ars alb 30C treated gills of *D. rerio*

S. No.	Peak posi	tion					Vibrational assignments	
	Control	1g	2g	3g	4g	5g	6g	
1	3401	3287	3293	3285	3293	3293	3348	amine N-H stretch
2	2922	2920	2923	2923	2923	2924	2924	CH ₃ asymmetric stretching: mainly lipids
3	2853	2851	2852	2853	2853	2853	2853	CH ₂ symmetric stretching: mainly lipids
4	1637	1637	1646	1641	1641	1647	1649	amide C=0 stretch
5	1542	1542	1545	1542	1542	1545	1541	amide II: N-H bending and C-N stretching of proteins
6	1414	1408	1404	-	-	-	-	N=0 bend
7	-	-	-	1152	1162	1165	-	C-N amines
8	1236	1232	1236	1235	1235	-	-	PO ₂ asymmetric stretching: mainly phospholipids
9	1050	1013	1058	1055	1055	1055	1051	C-C stretching of lipids

Ethyl alcohol, 4g and 2a4g for 7 and 14 d respectively, shows no significance changes in the position but the stretching of the peak was higher when compared to ars alb. Likewise when ars alb 200C (5g and 2a5g) and ars alb 30C (6g and 2a6g) alone given for 7 and 14 d respectively, shows the effect similar to arsenic intoxicated. Hence, it considered that higher the dilution was more effective.

DISCUSSION

This FTIR study was based upon the toxicology effect of arsenic in fish gills. A study on biochemical changes in the kidney of *Labeo rohita* intoxicated with arsenic found that FTIR study can be applied for toxicology [12]. As well a related study of ars alb 30C and ars alb 200 was said to be more effective [13]. Further studies were carried out that the ars alb contains ultra high dilution of Arsenic trioxide was re-equilibrate upregulated genes at the time of oxidate stress by brought back expression levels [14].

Since arsenic was treat along with the water, there might be an alteration in the protein content of the fish, and there seems the intensity was decreased in amide bands [15]. Arsenic is a kind of metalloid, and it has the capability to have a strong interaction with thiol groups, thiol residues in proteins, regarding these functions it affects the structure of the molecules present in the gills [16].

Cakmak and his colleagues [17] studied the effect of nonylphenol, found the structural analysis and functional proteins based upon FTIR study. Based his way the investigation was applied to arsenic and alternative medicine on arsenic toxicity i. e Arsenicum album. Furthermore, by this examination on FTIR-a time of 14 d concentrate on it was found that the measure of protein and lipid content in the gill was radically changed in a brief time of days when contrasted with arsenic intoxicated, control and ars alb habituated.

CONCLUSION

Arsenicum album was unrevealed as a major homeopathic medicine against arsenic poisoning. By this investigation, it was concluded that the FTIR study on gills shows the alteration due to arsenic intoxication. In contrast, when the fish was treated with a higher dilution of arsenic that is ars alb 200C shows the most promising result, whereas ars alb 200C. Shows compatible results when compared to ars alb 200C. Since arsenic intoxication shows variable effects in biochemical constituents of gills, it is essential to finding a valuable resource to treat. In such condition, homeopathic treatment may be considered as an alternate means to handle.

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CONFLICT OF INTERESTS

Declared none

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