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Research Article

FT-IR STUDY OF THE EFFECT OF ALUMINIUM AND THE INFLUENCE OF DFO AND DFP ON THE BIO-CHEMICAL CHANGES IN THE KIDNEY OF INDIAN CIRRHINUS MRIGALA

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ABSTRACT
Aluminium is naturally occurring, most abundant element in the earth crust and becomes toxic elevated concentrations are introduced into the environment. Kidney is involved in detoxification and high affinity to removal and excretion of toxic substances circulating in the blood stream. The aim of the present work is to find out the effect of aluminium and the influence of DFO and DFP on the bio-chemical changes in the kidney of Indian *Cirrhinus Mrigala* using Fourier Transformation Infrared Spectroscopy. Various important features have been observed in FTIR spectra of aluminium intoxicated kidney of Indian *Cirrhinus Mrigala*, altered lipid and protein profile and increased amide II content, indicating an alteration in the lipid and protein profile leading to modification in membrane composition. Further it is observed that acute exposure to aluminium causes some alteration in protein and amide II and increased in coil structure of alpha helix. Chelating agent DFO and DFP reduces the bio-chemical content in the kidney of Indian Cirrhinus Mrigala. Both the chelating agents are based antidote for aluminium toxicity.

KEY WORDS: Aluminium, Kidney, Biochemical changes, FT-IR, DFO and DFP.

INTRODUCTION

Aluminium is an essential trace element involved in many biochemical processes[1]. Aluminium is a nutritionally and economically important culture fish, the acute and chronic aluminium exposures and the influence of chelating agent DFO and DFP[2] are reducing the body burden to prevent the accumulation of aluminium on the kidney of Indian Cirrhinus Mrigala. Kidney accumulated high amount of aluminium 106.52 µg for acute exposure and 35.50µg/g, 44.51 µg/g, 47.61µg/g and 112.50 µg/g for chronic exposure at 15, 30, 60 and 90 days respectively. Aluminium toxicity is could produced diseases such as excessive headaches, long-term memory, psychomotor speed, abnormal heart rhythm depression, dementia, speech disorders, numbness of the hands and feet, kidney failure. FT-IR spectroscopy is an important technique to study the cellular changes at molecular level in various biological samples[3]. It is a valuable technique due to its high sensitivity in detecting changes in the molecular constituent, such as lipids, proteins and nucleic acids, simultaneously by monitoring the different functional groups belonging to these biomolecules. The shift in the peak positions, bandwidths and intensities of the bands all give valuable structural and functional information, which may have diagnostic value. In the present study *Cirrhinus mrigala* was selected for experimental investigation because of fish is highly sensitive to various toxicants, it has fast breeding period, commonly available, commercially and nutritionally important, it has tolerate a wide range of water quality parameters and temperature varying from the optimum.

Deferoxamine (DFO) is strong Al (III) and Fe (III) chelator, and is used clinically to treat aluminium and iron overload diseases. DFO act as antioxidant role and it forms a complex with Al (III) to inactive Al (III). DFO can produce nitroxide free radical through one-electron oxidation reaction, and hence cause lipid peroxidation and enzyme activation, especially high concentration[4].

Deferiprone (DFP) is an orally active chelator. Deferiprone is an oral drug that chelates aluminium and iron and is used to treat thalassaemia major. Deferiprone to treat patients with iron overload due to blood transfusions in patients with thalassaemia, a genetic blood disorder that causes anemia, who had an inadequate response to prior chelation therapy[5]. Deferiprone is in clinical trials in the United States to treat Contrast-Induced Acute Kidney injury and to slow progression of Chronic Kidney Disease.

LETHALITY STUDIES

Experiments were conducted in the laboratory for 90 days in 20 liters plastic trough. Unchlorinated water (Ph is 8.2, alkanity is 408 mg/L, temperature 27±2 ^{o}c) was used as the test medium. *Cirrhinus mrigala* fingerlings of 4±1 cm and body weight 8±1 gm were used as testing organism. The fish specimens collected from the local pond were acclimatized in the laboratory condition for 15 days [6]. Median lethal concentration (LC_{50}) for 120 hours was determined by the method of Litchfield and Welcoxon (1949).The sub-lethal concentration of aluminium sulphate was prepared on the basis of ten times dilution of the LC_{50} value. For bio-accumulation study adequate acclimated *Cirrhinus mrigala* fingerlings were divided in to five groups of 15 each. Fingerlings of group -I were reared in metal free exposed to 17.3 ppm of aluminium for 14 days (acute) and 5.2 ppm of aluminium for 90 days (chronic) respectively. The groups 3 and 5 were again treated with chelating agent DFO for another 15 days subsequently. All the control and treated fingerlings were fed daily with oil less groundnut cake. At the end of the experimental periods, the fishes were subjected to estimation of aluminium in kidney tissue.

SAMPLING AND ANALYSIS

After dissecting the fish, removed kidney was first lyophilized and made in to fine powder by agate mortar and pestle. The powder sample and KBr again lyophilized in order to remove the moist bound water which might interfere with the measurement of the amide-I band. The powder samples 5gm mixed with 100gm of dry KBr and subjected to pressure 5x10 ^{6} Pa and made in to clear plate of 13mm diameter and 1mm thickness. Absorbance spectra were recorded at room temperature (25 ± 1) ^{0}C in the 4000-400 cm^{-1} region using Nicolet Avatar -360 FT-IR spectrometer equipped with KBr beam splitter and an air-cooled DTGS

(deuterated triglycine sulfate) detector installed at Centralized Instrumentation and Services Laboratory (CISL), Annamalai University. For each spectrum, 128 scans were co-added at a spectral resolution of 4 cm^{-1}.The frequencies for all sharp bands were accurate to 0.01 cm -1.The absorption intensity of the peaks was calculated by the baseline method. Each observation was confirmed by taking three replicates. By ORIGIN 6.1 software, we analyzed the FT-IR spectra of Kidney.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software program, version 17. The results were expressed as mean ± standard deviations. The data were analyzed by analysis of variance (ANOVA). Probability level (*p*-value) of less than 0.05 was considered statistically significant.

RESULT AND DISCUSSION

The control group samples are compare to aluminium (Al) treated, aluminium (Al) + deferiprone (DFP) treated, aluminium (Al) +deferoxamine (DFO) treated then from the output graphs gave us the slightly difference between acute and chronic exposed of kidney tissues of *Cirrhinus mrigala* in the range of 4000 cm^{-1} to 400 cm^{-1} and variation of functional groups due to the presence of proteins, lipids, sacharides etc. Wavenumber vs. absorbance plots gave the peak values and assignment of functional groups and changes the Wavenumber gave the different stretching. Acute effects are rapidly occurs and expose the kidney where as chronic effects are long term and slowly expose the kidney.

Table-1 should give acute effects where the 50% fishes died in the time interval between 96 hours to 14 days, as compare Al treated, AL+DFO treated and AL+DFP treated to the control group samples , in Aluminium intoxicated, Al + DFO and Al + DFP treated kidney tissues of *Cirrhinus mrigala* fingerlings exposed to chronic concentration (17.3 ppm) gives as in the region of 3417 cm^{-1} of high area where Al treated compare to control is shifted to 0.17% and Al+DFO treated is shifted 0.49% and Al+ DFP treated is remain constant due to amide A mainly N-H stretching of protein with negligible contribution from O-H stretching[7]. In the range of 2958 cm^{-1} Al+DFO treated is changes to 0.13% and Al+ DFP treated is shifted to decrease by 0.06% but Al remain constant due to CH_3 asymmetric stretch mainly lipids and proteins[8]. In the range of 2925 cm^{-1} Al+DFO treated is shifted by 0.03 % but Al and Al+ DFP treated remains unchanged due the presences of CH_2 asymmetric stretch mainly lipids and proteins[8]. In frequency 1650 cm^{-1} Al treated and Al+DFP treated are same but Al+DFO treated shifted by 0.12% due to the presence of Amide I mainly C=O stretching of proteins that means in this frequency region the altered the alpha helix structure of protein[9]. At frequency range of 1542 cm^{-1} Al+DFO treated is shifted by 0.12% but Al+DFP treated and Al treated vibrational levels of frequencies are same due to the Amide II mainly N-H bending or C-N stretching of proteins[10]. In the range of 1454 cm^{-1} Al treated and Al+ DFP treated are changes by0.03% and 0.82% but Al+DFO treated remains same due the CH_3 asymmetric bending mainly proteins[11]. In the range of 1398 cm^{-1} Al treated is shifted by 0.14%, Al+DFO treated is shifted by 0.07% and Al+ DFP treated is shifted by 0.21% due the CH_3 symmetric bending mainly proteins[12]. In the range of 1236 cm^{-1} Al treated is remain constant , Al+DFO treated is change by 0.40% and Al+ DFP treated is shifted by 0.08% due PO_2- asymmetric stretching mainly Amide II[13]. In the range of 1079 cm^{-1} Al treated is change by 0.18% and Al+DFO treated is shifted by 0.37% but Al+ DFP treated is change by 0.27% due the CH_3 symmetric bending mainly proteins[14] as shown in fig 1. It is cleared that the average FT-IR spectra and biochemical changes of amide and proteins in fingerlings as shown in table-2. Similarly here the chronic effect occurs when the chemical produces deleterious effects more often they are a consequence or repeated long interval time. The most common effect of chronic effects is behavioral, physiological, biochemical and histochemical changes.

Table-3 should gives the explanations of the following as compare of control, aluminium intoxicated, Al + DFO and Al + DFP treated kidney tissues of *Cirrhinus mrigala* fingerlings exposed to chronic concentration (5.2 ppm) gives as in the region of 3420 cm^{-1} region Al intoxicated compare to control is 1.92% difference whereas Al+DFO is -0.07% and Al+ DFP is 0.08% due to amide A mainly N-H stretching of protein with negligible contribution from O-H stretching and the area of peak is also increases that means the Al+ DFP is not lower the amide level as shown in table-4. In the range of 2962 cm^{-1} Al+DFO treated and Al+ DFP are changes by 0.33% and 0.16 but Al treated is decrease by 0.20 due to CH_3 asymmetric stretch mainly lipids and proteins. In the range of 2926 cm^{-1} Al treated is reduced by 0.03 % but Al+DFO and Al+DFP remains unchanged due the presences of CH_2 asymmetric stretch mainly lipids and proteins. In frequency 1652 cm^{-1} Al is decrease by 0.12% and Al +DFP is also increase by 0.12% but Al+DFO is increased by 0.18 due to the presence of Amide I mainly C=O stretching of proteins that means in this frequency region the altered the alpha helix structure of protein. The band centered at 1545 cm^{-1} corresponds to the Amide II mainly N-H bending or C-N stretching of proteins and almost all vibrational levels of frequencies are same. In the range of 1457 cm^{-1} Al treated is change by 0.13%, Al+DFO is Change by - 0.06% and Al+DFP remain constant due the CH_3 asymmetric bending mainly proteins. In the range of 1398 cm^{-1} Al treated is increased by 0.14% and Al+DFO is increase by 0.07% , Al+DFP is change by 0.21% due the CH_3 symmetric bending mainly proteins. In the range of 1234 cm^{-1} Al treated is increased by 0.16% , Al+DFO is increase by 0.24% and Al+DFP is also increased by 0.08% due PO_2- asymmetric stretching mainly Amide II. In the range of 1084 cm^{-1} Al treated is and Al+DFO treated are remain constant but Al+DFP is decreased by 0.05% due the CH_3 symmetric bending mainly proteins as shown in fig 2.

The ratio of the absorption intensities of selected bands of the kidney tissue is given in table 2 for acute exposure. According to table- 5 the ratio of the peak intensities of the bands at ~ 1540 cm^{-1} and at 3286 cm^{-1} for control, aluminium intoxicated Al + DFO and Al + DFP treated Kidney tissues were 0.62, 0.46, 0.56, and 0.55. This corresponds to the changes of 25.8 %, 17.8%, and 16% due to aluminium intoxicated, Al +DFO and Al +DFP treatment respectively. In the chronic exposure the ratios are 0.62, 0.36, 0.42, and 0.47 for control, aluminium intoxicated, Al+DFO and Al+ DFP treated tissues respectively and to the change of responds 41.9%, 14%, and 23% due to aluminium intoxicated, DFO and DFP treatment respectively. These results suggest that the relative concentration of protein to water in the tissue membrane is considered lower in aluminium intoxicated, tissue compared with that of the control. For acute exposure the ratio of the intensity of the absorption of bands between the methyl band and the methylene band (I_{2956}/ I_{2854}) for the control, aluminium intoxicated, Al+DFO and Al+DFP treated tissue are 0.50,

0.46, 0.48 and0.47 which corresponds to the change of 8%, 4.2% and 2% with respect to aluminium intoxicated Al+DFP treatment respectively. Similarly in chronic exposure these ratios are 0.50, 0.25, 0.27 and 0.33 for the control, aluminium intoxicated, Al+DFO and Al+DFP treated which corresponds to the change of 50%, 7% and 24% due to aluminium intoxicated, Al+DFO and Al + DFP treatment respectively. The decrease in the ratios indicate a decrease in the number of methyl groups in the aluminium intoxicated tissues compared to that of methylene groups in protein fibers.

For acute exposure, the ratio of the intensities of the bands at ~1540 cm-1 and ~1651 cm-1 decrease from 0.48 for the control to 0.42 for aluminium intoxicated tissues. But in the presence of the chelating agents DFO and DFP, the ratios are 0.47 and 0.46 which corresponds to the change of 12.5% and 10.6% due to aluminium intoxicated, Al + DFO and Al + DFP treatment respectively. In chronic exposure then ratios are 0.48, 0.29, 0.30 and 0.40 for the control, aluminium intoxicated, Al + DFO and Al+ DFP treated which corresponds to the change of 39.5%, 3% and 27.5% due to aluminium intoxicated, Al+DFO and Al+DFP treatment respectively. These decrease ratios in the aluminium intoxicated tissue reflects an alteration in the level of proteins due to the aluminium intoxicated. For acute exposure, the ratio of the peak intensities of the bands at ~1080 cm-1 and ~1540 cm-1for acute exposure was found to be 0.61, 0.40, 0.43 and 0.57 for the control, aluminium intoxicated, Al+DFO and Al+DFP treated kidney tissues respectively which corresponds to the changes of 34%, 6.9% and 29% due to aluminium intoxicated, Al+DFO and Al+DFP treatment respectively. Also in chronic exposure the ratios are 0.61, 0.36, 0.40 and 0.47 for the control, aluminium intoxicated, Al+DFO and Al+DFP treated tissues which corresponds to the change of 40.9%, 10% and 23 % due to aluminium intoxicated, AL+DFO and AL+DFP and treatment respectively. These variations indicate a decrease in glycoprotein content in the aluminium intoxicated kidney tissues[15] as shown in table- 5.

CONCLUSION

It has been concluded from the above result; heavy metals primarily affect protein structure and induced the stress in the organisms. The present study show the DFO and DFP are the effective chelating agents for removing the aluminium in *Cirrhinus mrigala* and others living organisms, but chelating agent DFO was effective as compare DFP. In kidney tissues the aluminium causes was more as compare to brain and liver and alter the protein structure .The variation of acute and chronic exposures in glycoprotein content in the aluminium intoxicated kidney tissues and change the biochemical contents like protein and amide in *Cirrhinus mrigala*. Chelation therapy widely used in the management of metals poisoning and find out the lethal effects of the particular chelator. FT-IR study is an important tool to find out heavy metal intoxicated and cure study using DFO, DFP and the biochemical changes inside body of *Cirrhinus mrigala*.

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TABLE-1:FT-IR SPECTRA VIBRATIONAL ASSIGNMENTS OF CONTROL, ALUMINIUM INTOXICATED, AL + DFO AND AL + DFP TREATED KIDNEY TISSUES OF *CIRRHINUS MRIGALA* FINGERLINGS EXPOSED TO ACUTE CONCENTRATION 17.3 PPM.

Control	Aluminium intoxicated	Al+ DFO	Al+ DFP	Peaks Assignments
3417	3423	3434	3417	Amide A :mainly N-H stretching of protein with negligible contribution from O-H stretching
2958	2958	2962	2956	CH_3 asymmetric stretch: mainly lipids and proteins
2925	2925	2926	2925	CH_2 asymmetric stretch: mainly lipids and proteins
2923	2890	2927	2923	Amide I:C=O stretching of proteins
2856	2890	2883	2856	Amide II: N-H bending/C-N stretching of proteins
1650	1650	1648	1650	CH_3 asymmetric bending mainly proteins
1542	1542	1544	1542	CH_3 symmetric bending mainly proteins
1454	1456	1454	1442	PO_2^- asymmetric stretch: mainly Amide II
1398	1400	1397	1395	COO^- symmetric stretch: glycogen and nucleic acids
1236	1236	1241	1237	Amide A :mainly N-H stretching of protein with negligible contribution from O-H stretching
1079	1081	1083	1082	CO-O-C asymmetric stretch: glycogen and nucleic acids

TABLE 2: THE AVERAGE FT-IR SPECTRA OF THE CONTROL, ALUMINIUM INTOXICATED, AL+ DFO AND AL+ DFP TREATED KIDNEY TISSUES OF *CIRRHINUS MRIGALA*.

Bands	Control	Bands	Aluminium intoxicated	Bands	Al+ DFO	Bands	Al+ DFP
3417	132.88	3423	86.489	3443	0.093	3445	0.204
2958	1.815	2958	0.365	2954	73.375	2957	57.243
1650	22.502	1650	17.412	1648	0.143	2364	0.296
1542	4.701	1542	5.416	1544	2.572	1650	13.639
1236	1.360	1236	1.109	1159	0.047	1396	0.890
1079	5.951	1057	0.122	1062	0.144	1081	0.432

TABLE-3: FT-IR SPECTRA VIBRATIONAL ASSIGNMENTS OF CONTROL,ALUMINIUM INTOXICATED, AL + DFO AND AL + DFP TREATED KIDNEY TISSUES OF *CIRRHINU MRIGALA* 5.2 PPM.

Control	Aluminium intoxicated	Al+ DFO	Al+ DFP	Peaks Assignments
3332	3326	3396	3309	Amide A :mainly N-H stretching of protein with negligible contribution from O-H stretching
2957	2958	2956	2958	CH_3 asymmetric stretch: mainly lipids and proteins
2907	2908	2903	2905	CH_2 asymmetric stretch: mainly lipids and proteins
2857	2862	2856	2852	Amide I:C=O stretching of proteins
1656	1660	1650	1648	Amide II: N-H bending/C-N stretching of proteins
1544	1548	1542	1544	CH_3 asymmetric bending mainly proteins
1459	1462	1454	1458	CH_3 symmetric bending mainly proteins
1398	1399	1399	1396	PO_2- asymmetric stretch: mainly Amide II
1236	1237	1235	1236	COO^- symmetric stretch: glycogen and nucleic acids
1031	1035	1034	1032	Amide A :mainly N-H stretching of protein with negligible contribution from O-H stretching
873	881	874	875	CO-O-C asymmetric stretch: glycogen and nucleic acids

TABLE 4: THE AVERAGE FT-IR SPECTRA OF THE CONTROL, ALUMINIUM INTOXICATED, AL+ DFO AND AL+ DFP TREATED KIDNEY TISSUES OF *CIRRHINUS MRIGALA* AT CHRONIC CONCENTRATION

Bands	Control	Bands	Aluminium intoxicated	Bands	Al+ DFO	Bands	Al+ DFP
3334	0.090	3326	215.610	3326	4.679	3309	67.151
2923	11.902	2920	7.239	2922	33.298	2921	7.424
2852	4.313	2844	1.427	2842	10.133	2850	1.697
1656	54.249	1648	2.801	1654	1.324	1652	0.781
1544	9.929	1543	96.720	1542	1.453	1540	2.938
1459	3.317	1458	2.681	1456	2.201	1455	2.443
1230	9.038	563	4.147	1454	1.537	1236	1.722
1081	16.448	1079	1.040	1079	2.941	1080	1.761

TABLE-5: FT- IR absorption intensity ratio of selected bands of kidney tissues of *Cirrhinus mrigala* fingerlings for acute and chronic exposure

Ratio of bands	Acute				Chronic			
	Control	Aluminium intoxicated	Al+DFO Treated	Al+DFP treated	Control	Aluminium intoxicated	Al+DFO Treated	Al+DFP treated
I_{1540}/I_{3286}	0.62	0.46	0.56	0.55	0.62	0.36	0.42	0.47
I_{2956}/I_{2854}	0.50	0.46	0.48	0.47	0.50	0.25	0.27	0.33
I_{1540}/I_{1651}	0.48	0.42	0.47	0.46	0.48	0.29	0.30	0.40
I_{1080}/I_{1540}	0.61	0.40	0.43	0.57	0.61	0.36	0.40	0.47

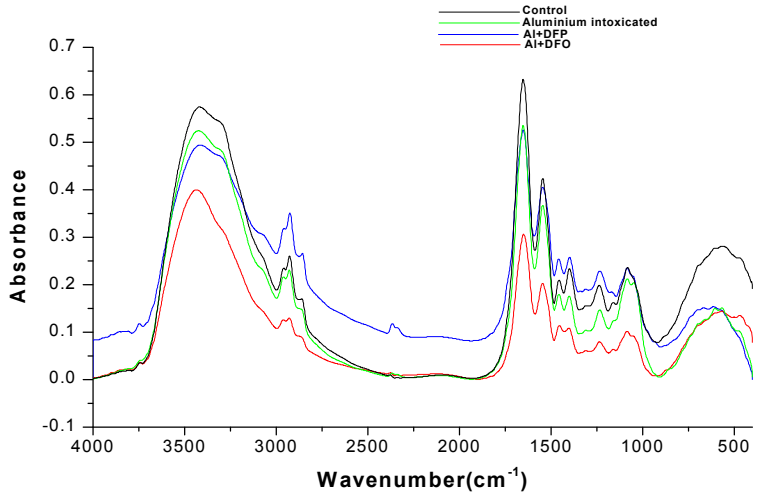

Fig-1: FT-IR spectra of control, Aluminium intoxicated, Al+DFO treated, Al+ DFP treated of Kidney tissues of *Cirrhinus mrigala* fingerlings for acute exposure 17.3PPM.

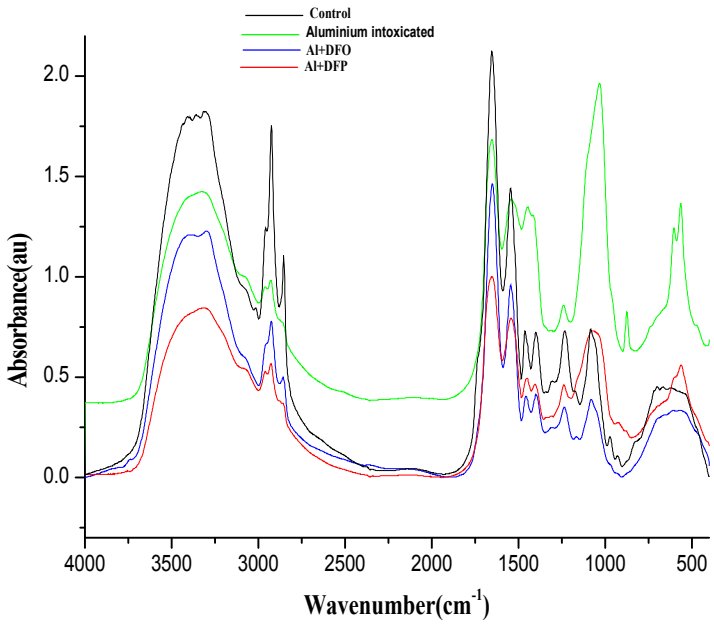

Fig 2: FT-IR spectra of control, Aluminium intoxicated, Al+DFO treated, Al+DFP treated of Kidney tissues of *Cirrhinus mrigala* fingerlings for chronic exposure at 5.2 PPM.

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