

RESEARCH ARTICLE

Evaluation of Hepatotoxicity of Lanthanide Complexes of Fish *Catla catla*

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Manuscript Details	ABSTRACT
<p>Received : 31.07.2014 Revised : 31.10.2014 Revised Received : 11.11.2014 Accepted: 13.11.2014 Published : 18.11.2013</p> <p>ISSN: 2322-0015</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Meshram Nitin P and Janbandhu Kishor S. Evaluation of Hepatotoxicity of Lanthanide Complexes of Fish <i>Catla catla</i>, Int. Res. J. of Sci. & Engg., 2014; 2 (6): 219-225.</p> <p>Copyright: © Author(s), This is an open access article under the terms of the Creative Commons Attribution Non-Commercial No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p>A series of rare earth complexes are being used for various medical uses particularly in the tumorology, immunoresonance transfer mechanisms, diagnosis of mutation and probing of Molecular interactions. Most of workers discussed the biodistribution and dosimetry for therapy planning of specific tumors related to stomatostatin receptor. Chelating complexes of various lanthanide series interact with biological tissue matter differently and may show highly specific responses with respect to tissue atrophy, tissue modification, antihaemolytic and even prevention of intravenous blood clotting.</p> <p>Terbium chelates have been selectively used in sensitized emission immunoresonance energy transfer (SEIRET) method, which can detect the association of molecules of Duchenne muscular dystrophy gene. Europium (III) has been successfully used as typical biomarkers because of their selective uptake and binding.</p> <p>The exposure of fishes to low doses of La and Nd complexes provided significant information about the low hepatotoxicity of such complexes. Increased levels of SGPT are indicative of hepatitis, obstructive Jaundice, Metastatic carcinoma, hepatic cojection and myocardial infarction. So that measurement of SGPT usually reflects liver status.</p> <p>Keywords: <i>A. flavus</i>, HBV, Aflatoxin, HCC, 16srRNA, Phylogenetic analysis.</p> <p>INTRODUCTION</p> <p>Chelating complexes of lanthanide (III) find variety of uses in biological science. Infact, a series of rare earth complexes are being ued for various medical uses particularly in the tumorology, immunoresonance transfer mechanisms, diagnosis of mutations and probing of molecular interactions (Douglas, 1997; Lopexz-Crapez <i>et al.</i>, 2001; Mathis, 1995; Leif <i>et al.</i>, 1976).</p>

Rosch *et al.* (2004) have reviewed the use of radio lanthanides in metal ions and their complexes while Foster *et al.* have discussed the biodistribution and dosimetry for therapy planning of specific tumors related to stomatostatin receptor (Rosch and Forssell-Aronsson, 2004).

Chelating complexes of various lanthanide series interact with biological issue matters differently and may show highly specific responses with respect to tissue atrophy, tissue modification, antihaemoytic and even prevention of intra enous blood clotting. Neodymium and Samarium nicotinate have been shown to posses anti blood clotting property by Chupa Khina *et al.* (1963). Similarly, Prata *et al.* (2002) have shown that D.T.P.. derivatives of lanthanides i.e. La (30 (+) and [3] [+]) show hepatobilliary specificity. Both in vivo and in vitro studies showed hepatophilic tendencies of these lanthanides.

The medicinal chemistry department and microbiology department of S.K. Porwal College, Kamptee is continuously producing organic chelating complexes of various rare earth elements. in our earlier studies with lanthanide complexes, anti haemolytic properties were noticed (Ghoshal, 1998).

In the present work, two organic chelates of lanthanum and Neodymium were provided for evaluating their hepatotoxicity in the fish (*Catla catla*). The aims and objectives of the present work are thus :

To rear fingerlings of *Catla catla* on a rearing tank along with proper maintenance of microbiological status of the tank.

To SGOT and SGPT levels as liver function markers in the reared fish *Catla catla*.

To study the histopathological status of hepatocytes of *Catla catla* treated with La and Nd complexes.

The tentative structures of the complexes under study are shown in Fig. 1.

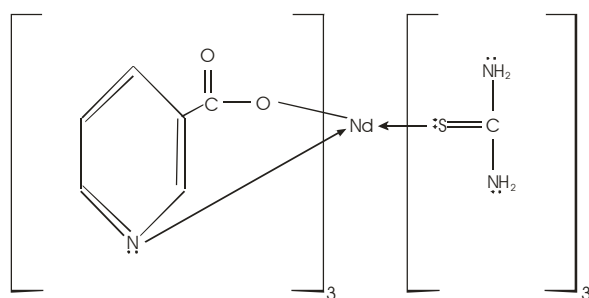


Fig. 1: Nd (NA)₃ (Tu)₃ Coordination Number - 9 Non Ionic Compound.

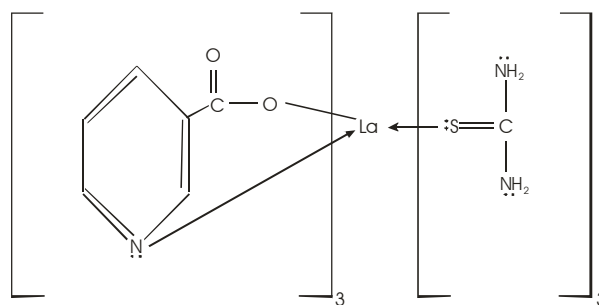


Fig. 2: La (NA)₃ (Tu)₃ Coordination Number - 9 Non Ionic Compound.

The complexes of rare earths are a series of compound which are becoming increasingly useful in treatment and diagnosis of many pathological conditions including tumor detection and cure immunodiagnosis of nucleic acid related assay techniques mostly involves biological samples that are dependent on fluorescent measurement. Such measurements are often confounded with limitations of sensitivity due to background signals, auto fluorescence or Raman scattering (Schwartz, 1993). The uses of assays based on complexes of lanthanide ion are becoming increasingly common.

Terbium chelates have been selectively used in sensitized emission immunoresonance energy transfer (SEIRET) method, which can detect the association of molecules of Duchenne muscular dystrophy gene. The dystrophin molecule selectively associates with actin component of the muscles which can be easily detected by SEIRET (Koenig *et al.*, 1988; Hammonds, 1987; Byers *et al.*, 1989; Ibraghimov, 1992).

Europium (III) has been successfully used as typical biomarkers because of their selective uptake and binding. Soini and Hemmila have very successfully utilized in multistep complexation the DELFIA technique (dissociation enhance lanthanide fluoro immune assay) (Soini and Hemmila, 1983) . Wyttenbach *et al.* (2004) have recently shown that lanthanide compounds la, Ce, Nd, Sm, Eu, Gd, Tb, Yb and Lu are selectively taken up by the leaves of six plants named Norway spruce, Silver fir, Maple, Ivy, Blackberry and Wood fern.

Rare earth metal ions are becoming useful probes in the measurements of rate of enzyme actions, Darnal and Birnbaum have shown that Nd(III)ion can greatly accelerate the rate of activation of the conversion of trypsinogen to trypsin. Similar activations are also reported in Ca(II) ion activation . Since, Nd(III) can be scrutinized by spectral and magnetic techniques, its use in enzyme biology and chemistry is evident (Darnal and Birnbaum, 1970).

The comprehensive uses of rare earth ion complexes such as phosphates, cryptates, platinum tetracyanide etc. are becoming, important tools in diagnosis. Moret et.al. have comprehensively dealt the use of rare earth complexes in biological sciences (Moret et al., 1991; Lauterbur et al., 1978; Evans, 1990).

MATERIALS AND METHODS

The present work included the following procedures which were carried out according to standard prescribed methods.

1. Rearing of fingerlings of *Catla catla*
2. Maintenance of microbiological status of the rearing tank
3. Acclimatization of the fishes in treatment aquarium tanks
4. Treatment of
5. La and Nd complexes
6. Hepatectomy and liver homogenization
7. Homogenate analysis
8. Histopathology (microtomy of liver)

Rearing

Small fingerling size fishes were borrowed from industrial fish and Fisheries department and reared in a tank of diameter 45 inches and height of 30 inches containing almost quarter volume of fresh water. Around 250 fingerlings were loaded in the water pretreated with KMnO₄. The fishes were fed with standard fish food initially continuously on a daily basis and later on in an interval of 3 to 4 days. On an average 5 to 8 buckets of water were replaced at an interval of 2 days. Total number of fishes died were also noted and intermittent doses of KMnO₄ was continued till the death of the fishes subsided. Such stabilized fishes were used for the experimentations. The tank photograph is shown in P3.1.

Maintenance of microbiological status

The fish tank was covered with mosquito net and regular replenishment of water was done to minimize microbial infections of the fishes. The tank was seeded with KMnO₄ to maintain constant D.O. levels. Maintenance is shown in P. 3.2.

Acclimatization :

Aquarium tanks of the size as shown in table 1. were set up. Each tank was fed with 4 liters of heavily oxygenated water. Requisite number of fishes were transferred from the rearing tank and allowed to

acclimatize before treatment. Aquarium tanks set up is shown in P. 3.3.

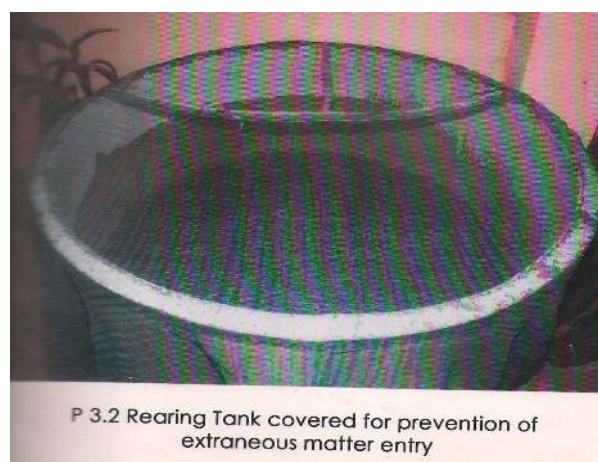
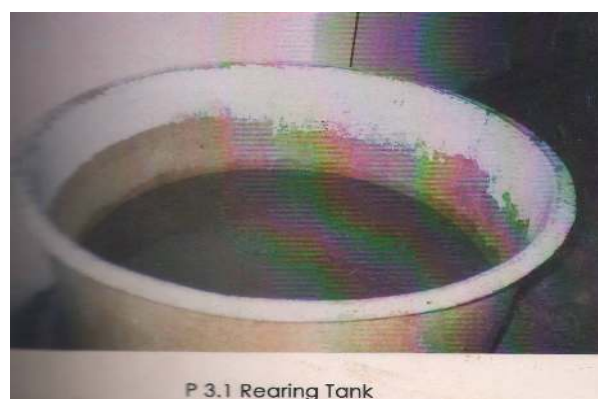


Table 1 : Dimensions of the aquarium :

Aquarium	Dimensions (in cm.)
A.	Length – 37.0 Breadth – 22.0 Height – 22.0
B.	Length – 30.7 Breadth – 21.5 Height – 22.0
C	Length – 37.0 Breadth – 19.5 Height – 19.5

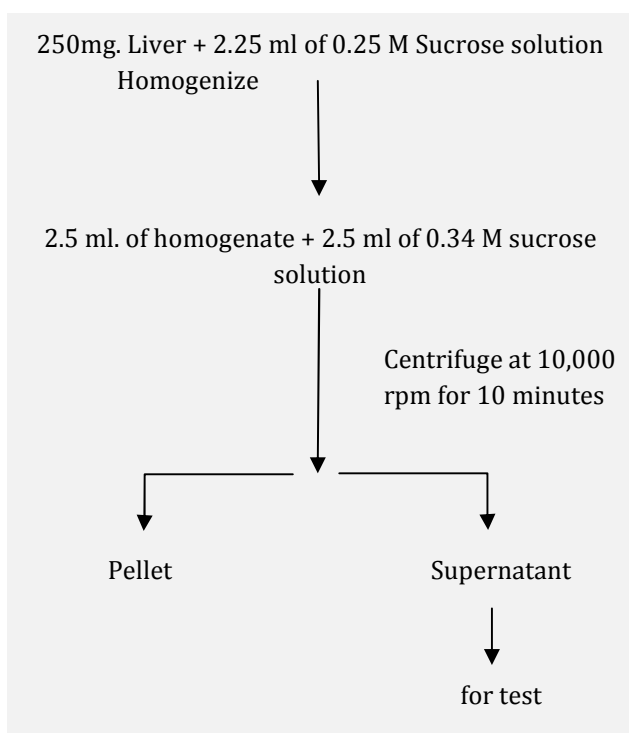
Treatment

33.3 and 66.6 mg. of La complexes were loaded per tank basis, while nd complexes were given at a dose of 100 mg. per tank. Each tank contains around 5 fishes exposed to the complexes for 7 days. The second experiment was conducted with 12 days retention time.

Hepatectomy and homogenization.

Fish liver was removed by lateral cuts as prescribed by the standard method of dissection of fishes. The liver pieces were divided into two sets, one for microtomy and the other for homogenization. The homogenization protocol is given below.

Protocol



Homogenate analysis

Homogenate supernatant was subjected to two types of analysis. A fraction of it was subjected to SGOT and SGPT as liver function test and the second fraction was sent to P.G.T.D. chemistry, R.T.M. Nagpur University for UV spectrophotometric analysis. SGOT and SGPT was performed by using beacon diagnostic kits as given below.

SGOT (AST)

(DNPH METHOD)

1. Plotting standard curve: The stock pyruvate standard should be used to plot the Calibration graph :

Pipette into test tubes	1	2	3	5	6
Enzyme activity units per ml.	0	24	61	114	190
Reagent 4 : pyruvate standard, 2mM, ml.	-	0.05	0.10	0.15	0.20
Reagent 1 : Buffered	0.50	0.45	0.40	0.35	0.30
Distilled water, ml.	0.10	0.10	0.10	0.10	0.10
Reagent 2 : DNPH colour reagent ml.	0.50	0.50	0.50	0.50	0.50
Mix well ; incubate at room temperature for 20 minutes					
Solution 1: Working reagent, ml.	5.0	5.0	5.0	5.0	5.0

mix well by inversion. Allow the tubes to stand at room temperature for 10 minutes. Read absorbance of the standard levels against distilled water at 505 nm or with a green filter.

2. Test procedure

Pipette into test tubes	Test (T)
Reagent 1 : Buffered Substrate	0.5 ml.
Incubate at 37°C for 5 minutes	
Sample	0.1 ml
Mix well, incubate at 37°C for 60 minutes	
Reagent 2 : DNPH colour reagent	0.5 ml.
Mix well, allow to stand at R.T. for 20 minutes	
Working reagent (Solution 1)	5.0 ml.
Mix well , Allow to stand at R.T. for 10 minutes	

Read absorbance of test at 505 nm or with green filter against distilled water.

SGPT (ALT)

(DNPH METHOD)

1. Plotting standard curve:

The stock pyruvate standard should be used to plot the calibration graph.

Pipette into test tubes	1	2	3	5	6
Enzyme activity units per ml.	0	28	57	97	150
Reagent 4 : Pyruvate standard, 2mM, ml.	-	0.05	0.1	0.15	0.20
Reagent 1 : Buffered Substrate, ml.	0.50	0.45	0.40	0.35	0.30
Distilled water, ml.	0.10	0.10	0.10	0.10	0.10
Reagent 2 : DNPH colour reagent ml.	0.50	0.50	0.50	0.50	0.50
Mix well; incubat at room temperature for 20 minutes					
Solution 1 : Working reagent, ml.	5.0	5.0	5.0	5.0	5.0

Mix well by inversion. Allow the tubes to stand at room temperature for 10 minutes. Read absorbance of the

standard levels against distilled water at 505 nm or with a green filter.

Plot a graph by putting absorbance on Y axis and known enzyme activity on X- axis to obtain test result of unknown samples.

2. Test procedure

Pipette into test tubes	Test (T)
Reagent 1 : Buffered Substrate	0.5 ml.
Incubate at 37°C for 5 minutes	
Sample	0.1 ml
Mix well, incubate at 37°C for 60 minutes	
Reagent 2 : DNPH colour reagent	0.5 ml.
Mix well, allow to stand at R.T. for 20 minutes	
Working reagent (Solution 1)	5.0 ml.
Mix well , Allow to stand at R.T. for 10 minutes	

Read absorbance of test at 505 nm or with green filter against distilled water

Histopathology :

The sections of the live were obtained by fixing them on wax blocks and microtomed fragments were fixed on the slides . The slides were stained as per the flow sheet shown below.

Staining Procedure

Xylene → Absolute alcohol → 90 % alcohol
 30 % alcohol ← 50 % alcohol ← 70 % alcohol
 Water → Hematoxyline → Water

70 % alcohol → 50 % alcohol ← 30 % alcohol
 Eosin → 70 % alcohol → 90 % alcohol
 Xylene ← Absolute alcohol

The fixed slides were treated with each of the chemicals for five minutes consecutively. the cover slip was then mounted with Canada balsam and permanent slide was obtained.

RESULTS AND DISCUSSION

Rearing characteristics

The fingerlings were reared in standard laboratory tanks as per the procedure described above. The standard informations about tank stabilization are shown in table 2.

SGOT and SGPT were determined from the control and treatment sets as per the protocol described above. The enzyme activity was compared in test and control and the activity ratio defined by .

$$\text{Activity Ratio} = \frac{\text{Activity in the test}}{\text{Activity in the control}}$$

The results are shown in table 3.

UV spectrophotometric analysis

All the supernatant samples were sent for UV analysis and no detectable La and Nd complexes were reported in any sample. The results are shown in table 4.

Table 2 : The standard informations about tank stabilization.

Month	Average Water replaced (Liters/ day)	Average death (No. of fishes / day)	KMnO4 frequency	Average length (incm.)
September , 10	4	3	2 per 5 days	4.5
October, 10	5	3	3 per 5 days	5.5
November 10	7	4	Alternate day	6.0
December , 10	10	2	Alternate day	7.0

Table 3: SGPT & SGOT activity ratio of test and control.

Enzyme	Treatment time (days)	Activity ratio		Nd
		La (33.3 mg)	La(66.6 mg)	
SGPT	7	1.185	1.194	1.101
SGOT		1.342	1.080	1.060
SGPT	12	1.379	1.120	2.064
SGOT		2.577	1.315	1.442
SGPT	Dead fish	-	1.601	-
SGOT		-	1.100	-

Table 4 : UV Spectrophotometric Analysis

Sr.No	Sample nature	Treatment days	Observation
1.	La(33.3 mg.)	7	La not found
2.	La (66.6 mg)	7	La not found
3.	Nd (100 Mg.)	7	Nd not found
4.	La (33.3. mg.)	12	La not found
5.	La (66.6 mg)	12	La not found
6.	Nd(100 mg.)	12	Nd not found
7	Dead fish La (66.6 mg.)	12	La not found

Histopathology

Histopathological slides were prepared and stained as per the procedure described above.

There was marked difference to both La and Nd treated hepatic cells. Results are shown in the table 5. and diagram. 4.1.

Table 5: Morphology of hepatic cells after treatment with La and Nd.

Sr. No	Nature of slide	Morphological observation
1.	Control	Normal hepatocytes with distinct nucleus with no contact aberration
2.	La(33.3. mg)	Cell shrinkage, diminished nuclei, occasional Kuffer cells
3.	La (66.6 mg.)	Cell shrinkage, diminished nuclei, Kuffer cells seen
4.	Nd (100 mg.)	Cell shrinkage, highly diminished nuclei, Kuffer cells seen

The exposure of fished to low doeses of La and Nd complexes provided significant informations about the low Hepatotoxicity of such complexes. Since, increased levels of SGPT are indicative of hepatitis, obstructive jaundice, metastatic carcinoma, hepatic conjestion, and myocardial infrctin, therefore, measurement of SGPT usually reflects live status and hence, it is called as liver function test. Although, lanthanum treated fished showed marginal increase in 7 days treatment with respct to SGPT but in showed significantly high level afte 12 days of exposure to La complexes. Similarly, the Nd complexes also showed considerable increase in SGPT levels in 12 days treatment as compared to 7 days treatment.

SGOT on the other hand is significant in the diagnosis of myocardial infraction and in explaining the cellular

permeability. Both La and Nd complexes affected SGOT levels and a similar increase can be seen after12 days of treatment as can be seen in table 5. Although mortablty rate of the fish in 12 days treatment was negligible but 1 fish died in the intermittent stage of treatment and the results of SGPT and SGOT of the said fish are shown in table 5.



Fig. 4.1(a): Atrophy of the Hepatic Cells

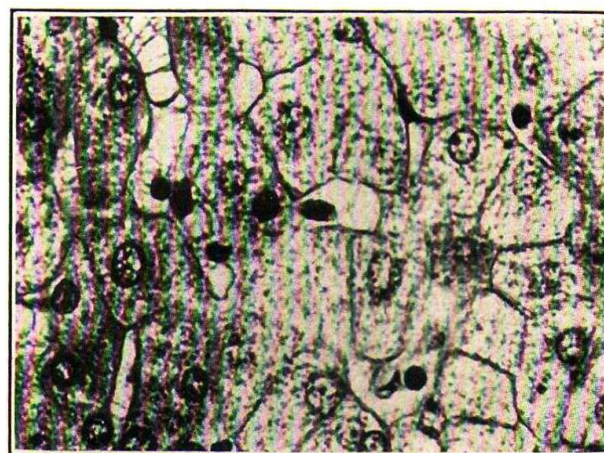


Fig. 4.1(b): Normal Hepatic Cells

That La and Nd complexes are partly hepatotoxic can be inferred from the hepatopathological examinations of their respective sections. Hepatobilliary constriction is very evident due to heavy shrinkage of the cells of the hepatocytes in both La and Nd treated samples of live cells. The diminished nuclei in all the cases suggest that these complexes might be interacting with specific proteins involved in chromaticn organization which needs to be further evaluated.

Since, the supernatant fraction did not show the presence of La and Nd complexes hence, it is plusibel that these complexes may be accumulative int eh liver cells itself, which needs further investigation.

CONCLUSION

The role of lanthanum and Neodymium complexes in the causation of hepatotoxicity is partly evaluated in this paper and the conclusions of the present work are

1. The levels of the liver function enzyme i.e. SGPT is increased in this fishes exposed to both La and Nd complexes.
2. The levels of hepatic SGOT is also increased in the fishes exposed to La and Nd complexes.
3. Aberrations in the hepatocytes are noticeable in the liver of the fishes exposed to La and ND complexes.
4. La and Nd complexes are not accumulating in the supernatant of liver homogenate.

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