Studies on Alkaline Phosphatase Levels of Fish Cyprinuscarpio Treated with Lanthanide Complexes.

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Abstract :-

The chelating complexes of lanthanide (III) having variety of uses in biological sciences. It is used in medical sciences particularly in Tumerology, Immunoresonance Transfer Mechanisms. Terbium chelates have been selectively used in the sensitized emission Immmuno resonance energy transfer method. Which can detect the association of molecules of duchenne muscular dystrophy gene.Eeropium III has been successively used as typical biomarkers because of their selective uptake and binding. So it is a fact the comprensive use of rare earth ions complexes such as phosphates, cryptates, platinum, tetracyanides are becoming important tools in diagnosis.

Keywords: Lanthanide Complexes , Alkaline Phosphatase ,Cyprinuscarpio

Introduction : -

Chelating complexes of Lanthanide (III) find variety of uses in biological science. In fact, a series of rare earth complexes are being used for various medical uses particularly in the Tumerology, Immunoresonance Transfer Mechanisms, Diagnosis of Mutations and probing of Molecular interaction(3,6,10,20).

Rosch et.al. have reviewed the use of radiolanthanides in metal ion and their complexes while Foster et.al have discussed the biodistribution and dosimetry for therapy planning of specific tumors related to stomatostatine receptor(8,9).

Chealting complexes of various lanthanide series interact with biological tissue matters differently and may show highly specific responses with respect to tissue atrophy, tissue modifications, antihaemolytic and even prevention of intravenous blood clotting Neodymium and Samarium, Nicotinates have been shown to possesanitiblood clotting property by Chupakhaina et.al. in 1963. Similarly hepatobilliary specifically. Both in vio van in vitro studies showed hepatophilic tendencies of these lanthanides(19).

We continuously producing organic chelating complexes of various rare earth elements. In our earlier studies with lanthanide complexes antihaemolytic properties were noticed(11,18).

In the preset work two organic chelates of Lanthanum and Neodymium were provided for evaluating their hepatotoxicity in the fish (Cyprinuscarpio). The aims and objectives of the present work are thus.

- To study the levels of alkaline phosphatase levels in the liver homogenates of fish (Cyprinuscarpio) treated with lanthanide complexes.
- To study the levels of alkaline phosphatase levels in the liver homogenates of fish (Cyprinuscarpio) treated with Neodymium complexes.
- Tentative structure of the complexes under study are shown in figure 1.1.

Literature Review

The complexes of rare earths are very useful in the field of medical sciences. A series of compounds which are becoming increasingly useful in treatment and diagnosis of many pathological conditions including tumor detection and cure.Immuno diagnosis 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, autoflurorescence or Raman scattering(21). The sue of assay based on complexes of lanthanide ions are becoming increasingly common.

Terbium chelates have been selectively used in the sensitized Emission Immuno Resonance Energy Transfer (SEIRET) method which can detect the association of molecules of duchenne muscular dystrophy gene. The dystrophin molecule selectively associate with action components of the muscles which can be easily detected by SEIRET(2,13,14,15).

Europium (III) has been successively used as typical biomarkers because of their selective uptake and binding. Soini and Hemmila have very successfully utilized in multistep complexation of Eu (III) to aminopolycarboxylate and b-diketonate ligands in the DELFIA technique (Dissociation Enhance lanthanide FluoroImmuno Assay)(7,12). 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 namely Norway spruce, silver fur, Maple, Ivy, Black berry and Woodfern(1).

Rare earth metal ions becoming useful probes in the measurements of rate of enzyme actions Darnall and Birnbaum have shown that Nd (III) ion can greatly accelerate the rate of activation of a 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(4).

The comprehensive use of rare earth ions complexes such as phosphates, cryptates, platinum, tetracynides etc. are becoming important tools in diagnoses. Moretet.al.,Lauterbut et.al and Evans et.al have comprehensively dealt the use of rare earth complexes in biological sciences(5,16,17).

Materials and Methods :-

Rearing and acclimatization of fishes

Figerlings sized fishes were obtained from Industrial Fish and Fisheries Department and reared on a labsize tank. Quarterfield with tap water and covered with mosquitonet. The tank was fead with standard fish food on daily bases. Three standard size aquarium with A length – 37 cm, breadth -22 cm, height – 22 cm; B Length – 30.7 cm, breadth – 21.5 cm, height 22 cm and C length – 37 cm, breadth – 19.5 cm, height – 19.5 cm were setup on the priority bases. On tank was kept as a treatment for Nd and the two tanks were treated with La of different concentration namely 33. mg/tank and 66.6 mg/tank and 66.6 mg/tank of La.

Nd100 mg corresponding controls were maintained and each tank was fead with set of five Cyprinuscarpio and kept for one week.

Hepatectomy

The fishes were cut from their anterior fronts and the liver was removed and immediately a part of it was processed for homogenization and the other part was kept at 4^{o}C for repeat analysis.

Liver Homogenization

250 mg liver + 2.25 ml of 0.25 M sucrose

Homogenise

2.5 ml of homogennate + 2.5 ml of 0.34 M sucrose

Centrigugate at 10,000 rpm for 10 min.

Pellet Supernatant

Mix well after addition of each reagents measure O.D of standard (S), Test (T) and control with distilled water to set zero at 510 nm. (Green Filter) (Table 1)

Experiments andResults :

All the Fishes treated with La and Nd. Complexes were subjected to hepatectomy along with control fishes. The liver homogenats of control as well as the treatment were subjected to homogenization and the liver homogenate was estimated for alkaline phosphatase activity. the result are shown in table 2 and 3

Table.1- Alkaline Phosphatase

Sr. no.	Reagents	Blank	Standard	Test	Control
1)	Carbonate buffer	1 ml	1 ml	1 ml	1 ml
2)	Substrate	2 drops	2 drops	2 drops	2 drops
Mix and incubate at 37^{o}C for 3 min.					
3)	Standard Phenol	-	0.1 ml	-	-
4)	Serum	-	-	0.05ml.	-
5)	4 – amino antipyrine	0.05 ml.	0.05 ml	0.05 ml	0.05 ml
6)	Serum	-	-	-	0.05 ml
7)	Poassiumferricyanide	0.5 ml	0.5ml	0.5ml	0.5ml

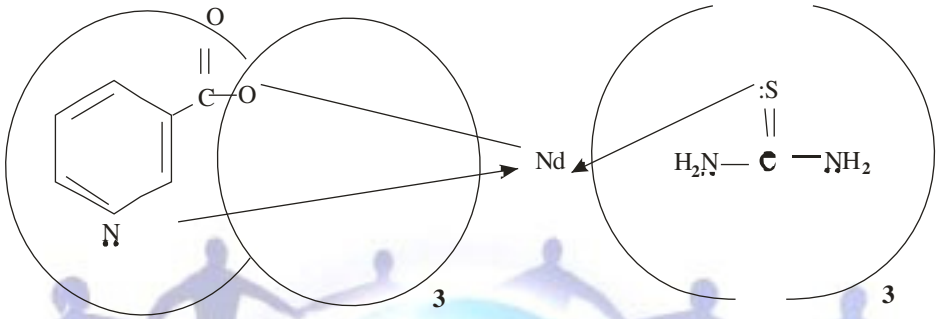
Table.2- Optical density variation of control and treatment

Set	Tanks	Blank	Standard	Test	Control
S1	A	1.678	1.888	1.592	1.587
	B	1.678	1.878	1.765	1.576
	C	1.294	1.934	1.620	1.515
S2	A	1.690	-	1.814	1.837
	B	Nd	Nd	Nd	Nd
	C	1.817	1.885	1.715	1.938

Nd = Not detected.

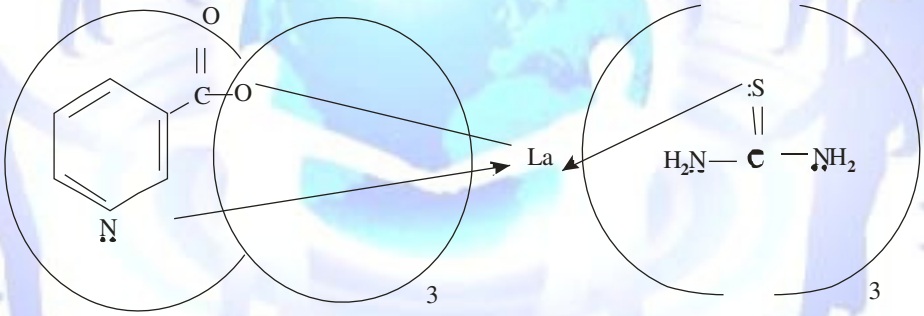
Table.3- Percent variations of Test and control

Set	Tanks	T/C x 100 = % variation
S1	A	(1.592/1.587) x 100 = 100.315 % variation
	B	(1.765/1.576) x 100 = 111.992 % variation
	C	(1.620/1.515) x 100 = 106.931 % variation
S2	A	(1.814/1.837) x 100 = 98.747 % variation
	B	-
	C	(1.715/1.938) x 100 = 88.493 % variation



$[Nd\ (NA)_3(TU)_3]$

Structure of Neodymium complex



$[La\ (NA)_3\ (TU)_3]$

Structure of Lanthanide complex

Figure. 1.1- Structure of NdAnd La Complexes

Discussion :

The liver homogenate was estimated for alkaline phosphate and no significant change could be observed in the complexes of lanthanum. However Nd complexes showed marginal elevation of these enzymes activities in the liver. Although ultraviolet absorption spectrophotometry results of the supernant done at PGTD chemistry R.T.M. Nagpur University showed no trace of these complexes indicating thereby that the complexes may be accumulation elsewhere or may be in the liver cells itself.

Partial changes in alkaline phosphatase indicates poor detoxification which may result in general debilitration of health of the fishes.

Conclusion :-

The present work entitled " Studies on Alkaline phosphatase levels of fish (Cyprinuscarpio) treated with lanthanide complexes." was a tentative investigative paper with respect to enzyme activity under the treatment of La and Nd complexes. Following conclusions are clear from the work :

- Levels of alkaline phosphatase increases not significantly in lanthanum but significantly in Nd complex treated fishes.
- These complexes of La and Nd are not accumulating in the supernatant of liver homogenate.

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