ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biological and Thermal Studies of Hetero-Metallic Mn -Ln Aggregates.

Muhammad Nadeem Akhtar [a], Zunaira Sultan[a], Muhammad Shahid[b], Syed Ahmad Tirmizi[c], Muhammad Asif Hanif[a] , Khaled F. El-massry[d1,2], and Ahmed H. El-Ghorab[d1,2*].

[a]Department of Chemistry, University of Agriculture, Faisalabad-38040, Pakistan.
[b]Department of Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan.
[c]Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan.
[d1]Faculty of Science, Aljouf University, Saudi Arabia.
[d2]Flavour and Aroma Department, National Research Center, Cairo, Egypt.

ABSTRACT

Thermal and biological studies of three heterometallic Mn-Ln polynuclear complexes (1-3) having general formula [MnIII2Ln3(nbdeaH)3(nbdea)2(piv)8]·MeCN, where bdeaH2 = N-butyldiethanolamine, piv = pivalate, Ln = Dy (1), Er (2) and Y (3) were done. The antioxidant activity of different hetero- metallic compounds was evaluated by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and enzyme inhibition studies were tested on acetylcholinestrase (AChE), butylcholinestrase (BChE), lipoxygenases (LOX) and tyrosinase. All complexes have shown low antioxidant activity while these complexes were showed significant activity against AChE and BChE. Moreover, complexes (1-3) showed low activities against the LOX and the tyrosinase. Thermal stability of these complexes was also studied and the thermal decomposition of compounds was interpreted in term of its structure and the end product was found to be metal oxide.

Keywords: Heterometallic Mn-Ln complexes, Acetylcholinestrase (AChE), DPPH and enzyme inhibition studies, Thermogravimetric studies.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In recent years interest in heterometallic complexes containing two different metal ions is fundamental not only for gaining insight in the structural but also have potential applications in molecular magnetism, catalysis, and biological systems etc [1,2,4]. The various metal ions are present in active sites of a number of metalloproteins and also in metalloenzymes like in tyrosinase and ureases [5] and these enzymes are involved in different biological processes. The biological activities of metal complexes differ from those of the metal ions or either ligands or biological activities may differ for various transition metal complexes [6]. Antioxidants are the compounds which terminate the attack of reactive species like free radicals and prevent it from different disease linked with oxidative damages inside the body [7].

The antioxidant activity of a synthetic compound can be measured by scavenging potential of that compound for the trapping of free radicals and these free radicals can oxidize biomolecules through nucleic acids, lipid, DNA, proteins, tissue damage and can initiate degenerative diseases. In addition, oxidative damage plays an important pathological role in various human diseases like arthritis, emphysema, cirrhosis atherosclerosis and cancer etc [8].

Almost all organisms are protected to some extent by free radical damaging enzymes like super-oxide dismutase or compounds just as ascorbic acid, phenolic acids, polyphenols, glutathione and flavonoids [9]. However, dietary antioxidants or antioxidant supplements may be sources of protection that the body needs to protect against the damaging effects of free radicals. [10] In Alzheimer's disease, the cholinergic system seemed susceptible to synapse loss [11].

Inhibition of acetylcholinesterase (AChE) has possible therapeutic applications in the treatment various diseases such as Parkinson's disease, myasthenia gravis and aging and mediated for the treatment of Alzheimer's disease. [12-14] In addition, butyrylcholinesterase (BChE) has been considered to be directly linked with the existing drugs for Alzheimer disease and the side effects of the acetylcholinesterase (AChE) inhibitors. Recently, studies indicated that AD plaques have higher quantities of BChE as compared in the plaques of age related non demented brains. Moreover, the further relevant studies showed AChE inhibitors are not linked with their poor selectivity toward AChE [15]. To overcome AD, AChE and BChE inhibitors were developed for the treatment and to restrain cholinergic function [16].

Tyrosinase is a multifunctional enzyme which contains copper and is found in animals and plants. Moreover, it is a critical enzyme that catalyzes both the ortho-hydroxylation of l-tyrosine and the subsequent conversion of 3,4-dihydroxyphenylalanine to DOPA-quinone in the beginning stage of melanogenesis. [17]. Tyrosinase is associated to pigmentation disorders in mammals and catalyzes the pigmentation of skin [18] and in vegetables this enzyme also causes a browning effect [19]. Moreover, the tyrosinase distributed in insects take part in host defense, wound healing, and the sclerotization process of insects [20].

Tyrosinase inhibitors have various potential applications such as in clinical medicine, food preservation technology and as bio-insecticides in agriculture [21,22]. The tyrosinase inhibitors studies have a much interest and many efforts have been done on the screening of efficient and safe tyrosinase inhibitors from synthetic materials and natural. Until now, various new tyrosinase inhibitors have been reported, but most of them have shown poor to moderate activity [23]. The different copper chelators act as tyrosinase inhibitors which have been reported and among the copper chelators, aromatic acids containing carboxylic groups shows strong inhibitory agents for tyrosinase due to the strong metal chelating ability with metal ions. Furthermore, research studies have been shown that some aromatic acids like benzoic acid, toluic acid, and Benzene-1,4-dicarboxylic acid, have been verified to be tyrosinase inhibitors [24].

LOXs are enzymes catalyze the formation of hydroperoxy derivates through oxygenation of polyunsaturated fatty acids. The multiple isoforms of LOXs are founds in plants which have various spatial and temporal distribution in plant development. The LOX studies were also linked with high temperature-induced damages both in root tissues and leaf [25]. Previously studies indicated that the elevated LOX activity enhanced lipid peroxidation and membrane damage in the presence of excess heavy metals in plant tissues. Further studies shows that enhanced lipid peroxidation through exposure of plants to heavy metals may be due to increase LOX activity which is involved in the initiation of oxylipin pathways. In Arabidopsis and

Phaseolus plants, after copper or cadmium treatment, jasmonic acid level increase and it might be due to the metal induced activation of LOX pathway [26-28].

Many researches has been done for synthesis of complexes based on 3d metal ions and their biological studies [29-30] but with respect to lanthanides compounds and its biological evaluation has been less studied [31]. Surprisingly bioinorganic chemistry Mn-Ln aggregates totally ignored with respect to biological studies.

In our previous work we have been published heterometallic compounds; [MnIII2Ln3(nbdeaH)3(nbdea)2(piv)8]•MeCN, where bdeaH2 = N-butyldiethanolamine, piv = pivalate, Ln = Dy (1), Er (2) and Y (3) [32] constructed by mixed ligands such a n-butyldiethanolamine and pivalic acid and their magnetic studies. Herein we are extending this research and now presenting biological studies such antioxidant, enzyme inhibition assay and thermal studies of Mn-Ln aggregates. To the best of our knowledge, no literature is currently available for mentioned biological evaluation of Mn-Ln aggregates.

EXPERIMENTAL

Materials

Manganese acetate, Hydrated Dysprosium nitrate, Hydrated Yttrium nitrate, Hydrated Erbium nitrate, Acetonitrile, N-butyldiethanolamine, Pivalic acid, Quercetin, Phosphate buffer (KH_2PO_4), 5´-Dithiobis-(2-Nitrobenzoic Acid), Kojic acid, Eserine, Baicalein. Complexes (1-3) were synthesized by reported procedure [32].

Synthesis of complex $[Mn^{III}_2Dy_3(^{n}bdeaH)_3(^{n}bdea)_2(piv)_8]\cdot MeCN$ (1)

A solution of $bdeaH_2$ (0.64 g, 4.0 mmol) in 10 mL of MeCN and $Dy(NO_3)_3\cdot 6H_2O$ (0.22 g, 0.5 mmol) was added to a stirred solution of $Mn(OAc)_2\cdot 4H_2O$ (0.12 g, 0.5 mmol) and pivalic acid (0.20 g, 2.0 mmol) in 10 mL of MeCN to give a dark-brown solution. After 48 h, brown crystals were collected by filtration and washed with acetonitrile.

Synthesis of complex $[Mn^{III}_2Er_3(^{n}bdeaH)_3(^{n}bdea)_2(piv)_8]\cdot MeCN$ (2)

Compound (2) is prepared with the same procedure as for compound (1) but $Er(NO_3)_3\cdot 6H_2O$ is used instead of $Dy(NO_3)_3\cdot 6H_2O$.

Synthesis of complex $[Mn^{III}_2Y_3(^{n}bdeaH)_3(^{n}bdea)_2(piv)_8]\cdot MeCN$ (3)

Compound (3) is prepared with the same procedure as for compound (1) but $Y(NO_3)_3\cdot 6H_2O$ is used instead of $Dy(NO_3)_3\cdot 6H_2O$.

Antioxidant activity by DPPH Assay

This method was carried out according to Noor et al. [33]. Ten µL of test solution was added in 96-wells plate followed by the addition of 90 µL of 100 µM methanolic DPPH solution in a total volume of 100 µL. The contents were mixed and incubated at 37°C for 30 minutes. The reduction in the absorbance was measured at 517nm using Synergy HT BioTek® USA microplate reader. Quercetin was used as standard antioxidant. All experiments were carried out in triplicates. For the determination of IC_{50} values, test solutions were assayed at various dilutions i.e. 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.015 mM. Data obtained was computed on Ez-fit software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula.

$$\text{Inhibition (\%)} = \left(\frac{\text{Abs. of control} - \text{Abs.of test solution}}{\text{Abs. of control}}\right)$$

Acetylcholinestrase (AChE) and Butylcholinestrase (BChe) Assays

The AChE& BChE inhibition activities were performed according to Ellman et al. [34] with slight modifications. In both assays total volume of the reaction mixture 100 µL contained 60 µL KH_2 PO_4 buffer (100 mM, pH 7.7). Ten µl test compound (0.5 mM per well) was added, followed by the addition of 10 µL enzyme. The contents were mixed, pre-incubated for 10 mins at 37ºC and preread at 405 nm. Then contents were pre-incubated for 10 mins at 37ºC. The reaction was initiated by the addition of 10µL of 0.5 mM per well substrate, followed by the addition of 10 µLDTNB (0.5mM per well). After 15 mins, absorbance was measured at 405 nm. All experiments were carried out with their respective controls in triplicate. For the determination of IC_{50} values, test solutions were assayed at various dilutions i.e. 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.015 mM. Data obtained was computed on Ez-fit software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula.

$$\text{Inhibition (\%)} = \frac{(\text{Abs. of control} - \text{Abs. of test solution})}{\text{Abs. of control}}$$

Where,

Absorbance of Control = Total enzyme activity without inhibitor
Absorbance of Test = Activity in the presence of test compound

Lipoxygenase (LOX) Assay

LOX activity was assayed according to the method described by Tappel [35] with slight modifications. A total volume of200 µlcontained 140 µl KH_2PO_4 buffer (100 mM, pH 8.0), 20 µl test compound and 15 µl purified LOX enzyme (600 units per well). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 minutes at 25°C. The reaction was initiated by the addition of 25 µl substrate solution. The change in absorbance was observed after 6-10 min at 234 nm. Baicalein (0.5 mM per well) was used as a positive control. For the determination of IC_{50} values, test solutions were assayed at various dilutions i.e. 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.015 mM. Data obtained was computed on Ez-fit software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula.

$$\text{Inhibition (\%)} = \frac{(\text{Abs. of control} - \text{Abs. of test solution})}{\text{Abs. of control}} \times 100$$

Anti-tyrosinase Assay

Anti-tyrosinase activity was assayed according to Lee [36] with slight modifications. Total volume of reaction mixture contained 100 µl,60 µl 100 mM phosphate buffer, pH 6.8, 10 µl mushroom tyrosinase enzyme (5 units) and 10 µl 0.5 mM test compound mixed in 96-well plate. Contents were preincubated for 5 minute at 37^{o}Ċ. After incubation, 20 µl of 10mM L-dopamine was added as a substrate. Contents were mixed and incubated for further 30 min. Absorbance was taken at 490 nm using Synergy HT BioTek 96-well plate reader. The enzyme inhibition (%) was calculated by the bellow formula:

$$\text{Inhibition (\%)} = \frac{(\text{Abs. of control} - \text{Abs.of test solution})}{\text{Abs. of control}} \times 100$$

Thermogravimetric analysis (TGA)

A Thermogravimetric (TG) analysis was conducted with METTLER TGA 851e under flowing N_2 stream (flow rate 40 mL/min) from room temperature to 800°C at a heating rate of 10 K/min.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Radical scavenging activity using DPPH

Free radical scavenging is one of the best known mechanisms by which antioxidants inhibit lipid oxidation. DPPH radical scavenging activity evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds or extracts [29].Antioxidant activity of complexes (1-3) as well as reference compounds is summarized in Table **1.** From the results, it is clear that the free radical scavenging activities of Mn-Ln complexes revealed that these complexes exhibit lower activity than those of quercetin (0.5mM). DPPH is a stable free radical that can accept an electron or hydrogen radical from ligand and thus be converted into a stable, diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm thus Mn-Ln complex was found to have slight antioxidant activity as shown in Table 1, indicating their abilities to act as radical scavengers.

Table 1: Antioxidant and Enzyme inhibition and their IC_{50} activities of the synthetic heterometallic compounds

Biological assay	Compounds			Standards	
DPPH assay		Activity (%)	IC_{50} (µmoles/L)	Activity (%)	IC_{50} (µmoles/L)
	1	22.08±0.14	-	93.21±0.97	16.96±0.14
	2	28.19±0.61			
	3	35.17±0.42			
AChE	1	95.58±0.85	52.81±0.14	91.29±1.17	0.04±0.001
	2	96.17±0.42	51.21±0.31		
	3	90.12±0.34	63.21±0.04		
BChE	1	73.19±0.69	101.21±0.11	82.82±1.09	0.85±0.001
	2	69.45±0.73	125.51±0.04		
	3	81.47±0.54	66.61±0.11		
LOX	1	33.01±0.96		93.79±1.27	22.4±1.3
	2	26.84±0.14			
	3	33.86±0.55			
Anti-tyrosinase	1	31.13±0.56		93.50±0.91	6.04±0.11
	2	34.60±0.98	Nill		
	3	27.88±0.84	Nill		

Standards used in the current study along with their concentration: Quercetin (0.5mM) for DPPH, Eserine (0.5mM) for AChE and BChE, Baicalein (0.5mM) for LOX and kojic acid (0.5mM) forAnti-tyrosinase.

Acetylcholinestrase (AChE) and Butylcholinestrase (BChe) Assay

The Mn-Ln complexes were studied for AChE and BChE inhibition activities from table **2** it was shown that all these complexes are active inhibitor AChE and BChE. They can be used for designing drugs, which control dementia more specifically and consequently more successfully.It has been shown that the amyloid protein precursor (APP) have specific selective metal binding sites which mediate its physicochemical behavior and if occupied, may cause inhibition of AChE and BChE. BChE inhibitors have been used to delay symptoms of AD patients by virtue of their ability to enhance acetylcholine availability. Thus, new metal based inhibitors for AChE and BChE are among the most sought after targets for therapeutic use in AD treatment [37-38].

Here, we have shown that these heterometallic complexes might be reasonable alternative drugs, which have been used for the inhibition of acetylcholinesterase butyrylcholinesterase in order to treat Alzheimer's disease and for related illnesses. Measurement of scavenging assay showed that all these hetero metallic complexes i-e $Mn^{III}_2Dy_3$**(1),**$Mn^{III}_2Er_3$ **(2),**$Mn^{III}_2Y_3$ **(3)** have inhibitory activity. It is concluded that the $Mn^{III}_2Y_3$ (**3**) has more AChE activity with IC_{50} value 63.21±0.04 µmoles/L and complex (**2**) [$Mn^{III}_2Er_3$] has more BChE activity with IC_{50} value 125.51±0.04µmoles/L.

Lipoxygenase (LOX) Assay

LOX is an enzyme that catalyzes the oxidation of arachidonic acid to leukotrienes, in an essential mechanism for cell life. Prostaglandins, the final products formed from the metabolism of arachidonic acid,

contribute to tumorigenesis as angiogenic factors. Studies have shown that LOX inhibitors induce the release of cytochrome c from mitochondria into the cytosol, causing apoptosis through the mitochondrial pathway both in vivo and in vitro.

LOX assay of complexes as well as reference compounds is summarized in Table **2.**The complexes were studied for LOX for a possible therapeutic activity for diseases such as bronchial asthma, inflammation and tumour angiogenesis. Result for LOX scavenging assay shows that all these complexes **(1-3)** have very small inhibitory activity as compared to the standard compound Baicalein (inhibitor of LOX).More importantly, the inhibitory activity of these complexes is closely related to their spatial arrangements, which determine their interaction with LOX. These complexes tend to bind competitively to LOX at the substrate binding site. The bulky metal complexes inhibit the enzyme activity.

Table 2: Thermal Stability of the synthetic heterometallic compounds using thermogravimetric analyses (TGA)

Compounds	TG range (°C)	Mass loss %	Total mass loss	Assignment	Final solid residue
(1)	59.11-176.38	16.05(15.82)	86.47%	MeCN, $bdeaH_2$	Dy_2O_3
	176.39-280.85	65.57(64.45)		Pivalic acid	
	280.86-549.26	4.85(6.94)		Mn	
(2)	140.13-288.31	1.725(1.8)	74.57%	MeCN	Er_2O_3
	288.32-435.42	4.622(4.95)		Mn	
	435.43-573.85	68.22(75.06)		Pivalic acid, $bdeaH_2$	
(3)	131.57-275.44	17.78(17.95)	86.90%	MeCN	Y_2O_3
	275.45-435.29	45.34(44.02)		Mn, Pivalic acid	
	435.30-573.85	23.81(28.64)		$bdeaH_2$	

Anti-tyrosinase Assay

Depigmentation agents act at various levels of melanin production in the skin. Many of them are known as competitive inhibitors of tyrosinase, which is the key enzyme in melanogenesis. This enzyme is a copper-containing enzyme that catalyzes the production of melanin from tyrosine by oxidation reaction. Hence, the compounds that inhibit tyrosinase activity leading to skin lightening have been the subject of much research.

Anti-tyrosinase activity of complexes as well as reference compounds is summarized in Table **2.** The complexes **(1-3)** are studied for anti-tyrosinase activities for a possible therapeutic activity for various diseases. It has been shown that the tyrosinase possesses specific selective active binding sites; if these active sites are occupied by metal chelaters then it may stop the activity of tyrosinase enzyme. The studies of tyrosinase scavenging assay shows that all these complexeshave low inhibitory activity as compared to the kojic acid (which is a tyrosinase inhibitor) which shows that these Mn-Ln heterometallic complexes have slight ability to inhibit tyrosinase or have slight anti-tyrosinase activity.

The formation of peptide or protein aggregates is a common feature of many different forms of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease and prion diseases. It is also widely accepted that various metal ions can play a significant role in the conformational changes of these peptides/proteins.

The antioxidant, LOX and anti-tyrosinase activities of the complexes were significantly low, so in the current study their IC50 were very high and cannot provide significant impact in the data. So there values are not presented in the table1.

ISSN: 0975-8585

Thermal study of (1-3) complexes

Thermal stability of **1-3** complexes was examined by the thermogravimetric analyses (TGA) under nitrogen atmosphere at the temperature range of zero to 800°C. Thermograms of compounds (Figures 1-3) show three stages of weight loss due to the elimination of various components at different temperatures is shown in following table. First weight loss of complex **1** is 16.05% (cal:15.82%) at the temperature 59.11°C-176.38°C due to elimination of MeCN and N-butyldiethanolamine. 65.57% (cal:64.45%) weight loss occur in second stage due to elimination of pivalic acid. In the third stage 4.856% (cal:6.9405%) weight loss occur due to the elimination of Mn at temperature 280.85°C-594.26°C. First weight loss of complex **2** is 1.725% (cal:1.8%) at the temperature 140.13°C-288.31°C due to elimination of MeCN. Weight loss in second stage is 4.622% (cal: 4.95%) due to elimination of Mn at temperature 291.32-435.42°C. In the third stage 68.22% (cal: 75.06%) weight loss occur due to the elimination of pivalic acid and N-butyldiethanolamine at the temperature 437.43-573.85°C .For complex **3** weight loss in first step 17.789% (cal :17.95) at the temperature 131.57-275.44°C due to removal of MeCN. The second weight loss is 45.34% (cal: 44.0169%) at temperature 275.46- 435.29°C due to the elimination of pivalic acid and Mn. The third weight loss is 23.81% (cal: 28.64%) at the temperature 435.30-573.85°C due to the removal of N-butyldiethanolamine. The overall weight loss in complexes 1, 2 &3 is 86.47%, 74.57%, 86.90% respectively leaving the metal oxides as residue which has higher boiling point. This implies that high coordination number and the coordination environment of lanthanide ions with ligand have remarkable effects on thermal stability.

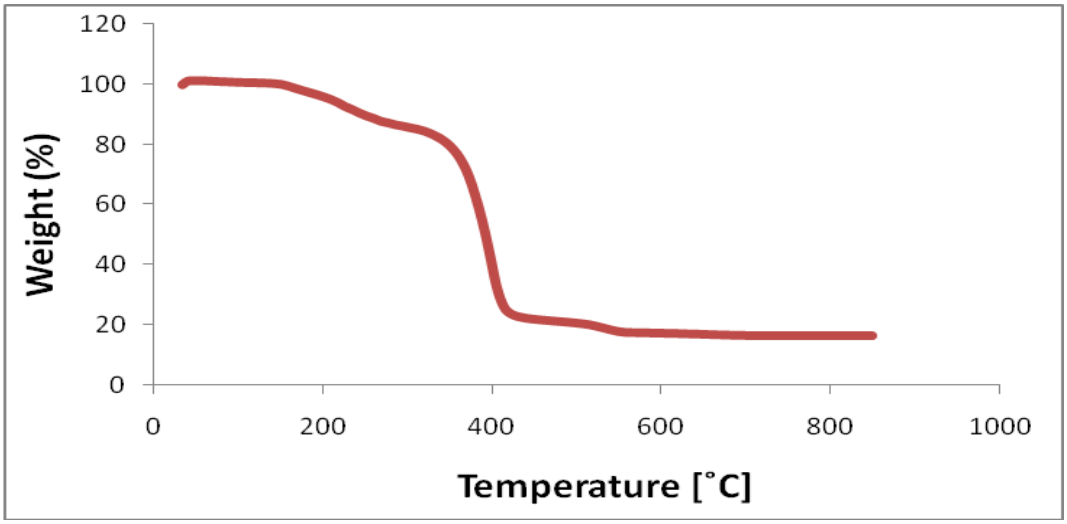

Figure 1: TGA curve of complex (1)

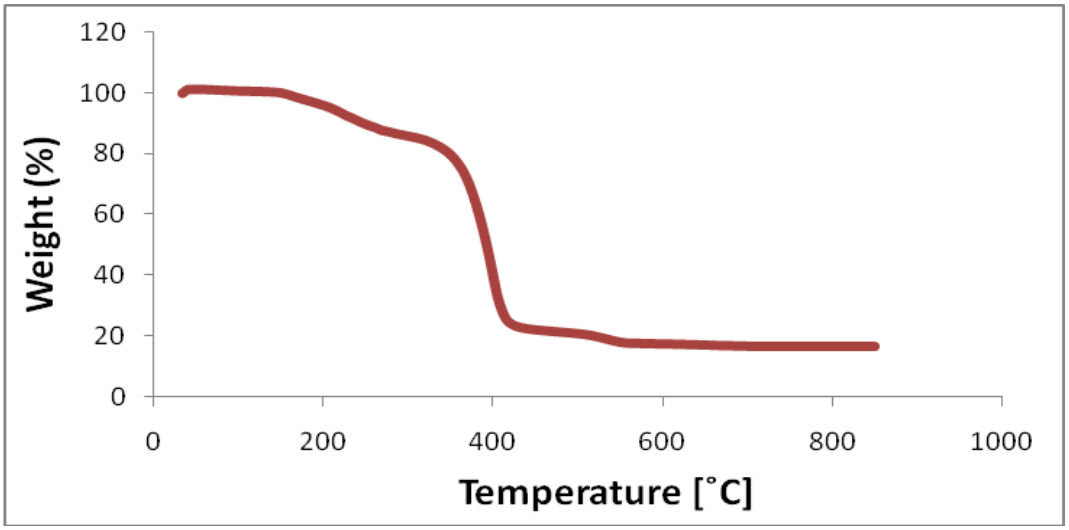

Figure 2: TGA curve of complex (2)

ISSN: 0975-8585

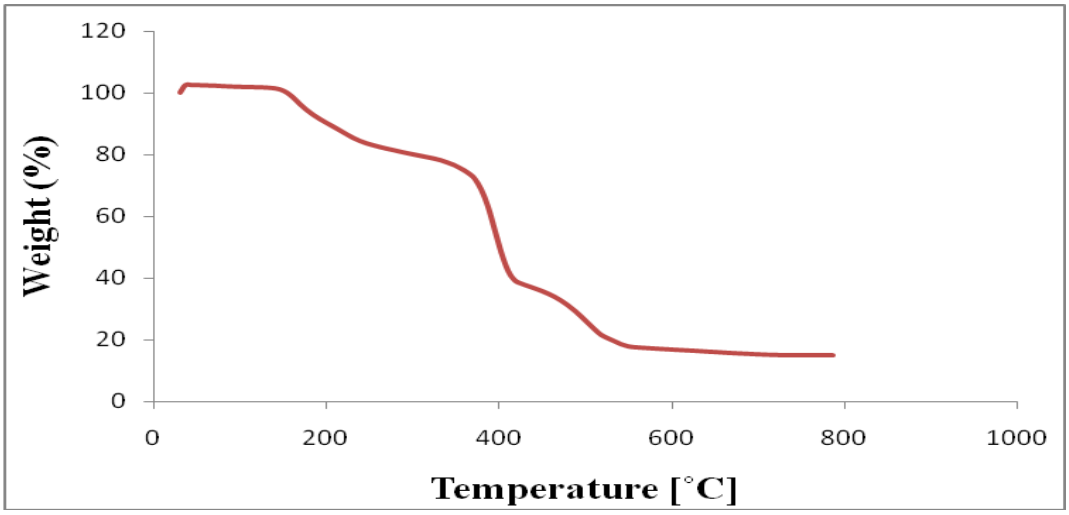

Figure 3: TGA curve of complex (3)

CONCLUSIONS

Thermal and biological studies of three heterothallic Mn-Ln complexes (1-3) were done and these complexes were found to have low antioxidant activity. The AChE and BChE inhibition activities show that all these complexes are active against AChE and BChE. It is concluded that the $Mn^{III}_2Y_3$(**3**) has more AChE inhibition activity and complex (**2**) $[Mn^{III}_2Er_3]$ has more BChE inhibition activity. In the enzyme inhibition selected complexes showed low activities against the LOX and the tyrosinase. Results of biological assays showed that **2** and **3** complexes have significant AChE and BChE inhibition activities, so it can be used as metal based drugs for Alzhemir disease after comprehensive screening. Thermal stability of **1-3** complexes was examined and the thermograms of compounds show mainly three stages of weight loss due to the elimination of various components at different temperatures. The end product was metal oxides as residue.

REFERENCES

[1] Cenini S, Galloa E, Caselli A, Ragaini F, Fantauzzi S, Piangiolino C. Coord Chem Rev 2006; 250, 1234–1235.
[2] Chan JN, Huang ZY, Merrifield ME, Salgado MT, Stillman MJ. Coord Chem Rev 2002; 233 319–339.
[3] Khan O. Struct Bond 1987; 68, 89- 117.
[4] Chan JN, Huang ZY, Merrifield ME, Salgado MT, Stillman MJ. Coord Chem Rev 2002; 319–339.
[5] Karplus PA, Pearson MA, Hausinger RP. Accounts of Chemical Research 1997; 30, 330.
[6] Krishnamoorthy P, Sathyadevi P, Cowley AH, Butorac RR, Dharmaraj N. Eur J Med Chem 2011; 46, 3376–3387.
[7] Rice-Evans CA, Miller NJ, Paganga G. Free Rad Biol Med 1996; 20(7), 933.
[8] Maxwell SR. Drugs1995; 49, 345.
[9] Niki E, Shimaski H, Mino M. Antioxidantism-free and biological defense, Gakkai Syuppn Center, Tokyo 1994; pp. 3-16.
[10] Prior RL, Cao G. Am J Nutraceut Assoc 1999; 2, 46.
[11] Bailey JA, Lahiri DK. J Neurochemistry 2010; 112, 4. 843–853.
[12] Sugimoto H, Ogura H, Arai Y, Iimura Y, Yamanishi Y. Japanese J Pharmacol 2002; 89, 7.
[13] Shankar GM, Li S, Mehta TH, arcia- Munoz GA, Shepardson NE, Smith I, Brett FM, arrell FM A, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ.. Nature Medicine 2008; 14, 837.
[14] Yu Q, Holloway HW, Utsuki T, Brossi A, Greig NH. J Medicinal Chemistry1999; 42, 1855.
[15] Greig NH, Utsuki T, Yu Q, Zhu X, Holloway HW, Perry T, Lee B, Ingram DK, Lahiri DK. Current Medical Research Opinion 2001; 17, 159.
[16] Mohamed T, Rao PPN. Bioorganic and Medicinal Chemistry Letters 2010; 20, 12, 3606–3609.
[17] Ito SY, Wakamatsu K, Ozeki H. Pigment Cell Res 2000; 13, 103–109.
[18] Ray K, Chaki M, Senqupta M. Prog Retin Eye Res 2007; 26, 323–58.

[19] Gandía-Herrero F, Jiménez M, Cabanes J, García-Carmona F, Escribano J. J Agric Food Chem 2003; 51:7764–9.
[20] Guerreo A, Rosell G. Curr Med Chem 2005;12:461–9.
[21] Gasowska-Bajger B, Wojtasek H. Bioorg Med Chem Lett 2008;18:3296–300.
[22] Friedman M. J Agric Food Chem1996;44:631–53.
[23] Shimizu K, Kondo R, Sakai K. PlantaMed 2000;66:11–5.
[24] Yin SJ, Si YX, Chen YF, Qian GY, Lü ZR, Oh S, et al. Protein J 2011;30:273–80.
[25] Ali, M.B., Hahn, E-J., Paek, K-Y. Plant Physiol. Biochem 2005; 43, 213-223.
[26] Maksymiec, W. Biol. Plant 2011; 55, 112-118.
[27] Cuypers, A., Smeets, K., Ruytinx, J., Opdenakker, K., Keunen, E., Remans, T., Horemans, N., Vanhoudt, N., Van Sanden, S., Van Belleghem, F., Guisez, Y., Colpaert, J., Vangronsveld, J.,. J. Plant Physiol 2011. 168, 309-316.
[28] A. Kulkarni, P. G. Avaji, G. B. Bagihalli, S. A. Patil, and P. S.Badami. Journal of Coordination Chemistry 2009, vol. 62, no. 3, pp. 481–492,.
[29] Jamil M, Zubair M, Asim Farid M, Altaf AA, Rasool Nasim NH, Ashraf M, Rashid MA, Abida Ejaz S, Yaqoob A, Ahmad VU, J Chem Soc Pak 2014; 36, 3 , 491-497.
[30] Pellerito L, Nagy L. Coordination Chemistry Reviews 2002; 224, 1–2, 111–150.
[31] Issa YM, Omar MM, Sabrah BA, Mohamed SK. Journal of the Indian Chemical Society. 1992;69:186–189.
[32] Akhtar MN, Lan Y, Mereacre V, Clerac R, Anson CE, Powell AK. Polyhedron 2009; 28, 1698–1703.
[33] Noor N, Raja Adil Sarfraz, Ali S, Shahid M. Food Chemistry. 2014; 143, 362–366.
[34] Ellman, GL, Courtney KD, Andres V, Featherstone RM. Biochem Pharmacol1961,7, 88-95.
[35] Tappel AL. Archieves of Biochemistry and Biophysics1953; 44(2): 378–395.
[36] Lee HS. J Agric Food Chem 2002; 50, 1400-1403.
[37] Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Food Chem 2004; 84, 551–562.
[38] Greig NH, Utsuki T, Yu Q, Zhu X, Holloway HW, Perry , Lee B, Ingram DK, Lahiri DK.. Curr Med Res Opin 2005;17: 159.