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Biochemical Activities of main Group Metal Chelates of Curcuminoid Analogues

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Abstract

Turmeric and its active chemical constituents, curcuminoids, have been reported to possess a number of medicinal uses particularly in the treatment of inflammation, angiogenesis, tumorigenesis, diabetes etc. Structurally, curcuminoids are 1,7-diaryl-1,6-heptadiene-3,5-diones and are known to form metal complexes similar to other 1,3diketones. It has been reported that metal complexation of these α, β,-unsaturated 1,3-diketones lead to dramatic changes in their biochemical activities including antitumour, antimicrobial and antioxidant activity. The two new curcuminoid analogues namely 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione and 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione have been synthesized and were complexed with main group metals Al(III), Ga(III) & In(III). They were characterized by IR, UV, ^{1}H NMR, ^{13}CNMR and Mass spectral techniques. *In vitro* cytotoxic studies were done with ligand and metal complexes (Al,Ga & In) against DLA and EAC cells using Tryptan blue exclusion method and antibacterial study of the compounds were done using agar well diffusion method. The *in vivo* antitumour activity of the ligand and complexes were determined by using DLA cells in mice and compared with standard anticancer drug cyclophosphamide.

Keywords: 1,7–diaryl heptanoids; in vivo antitumour; cytotoxicity: ascites tumour reduction

1. Introduction

Cancer is one of the most life –threatening diseases and cause serious public health problems in both developed and developing countries. It is a group of diseases characterized by the disregulate proliferation of abnormal cells that invade and disrupt surrounding tissues. Reactive Oxygen Species (ROS) play a key role in several normal physiological processes including chronic inflammation and cancer. Several structural classes of compounds are in use to defend against tumour; however they have severe side effects. Curcumin is one of the most potent and multi-targeting phytochemicals against a variety of cancers. Curcumin is a yellow pigment present in the Indian spice turmeric, the rhizomes of the traditional Indian medicinal plant (*Curcuma Longa* Linn). Turmeric has been used for centuries in Ayurvedic medicine. Based on this traditional usage dietary supplement containing turmeric rhizome and turmeric extracts are also being used nowadays. The curcuminoids occurring naturally in turmeric are curcumin, demethoxy curcumin and bis demethoxy curcumin. Curcumin has several biological effects exhibiting anti inflammatory[1-5] and antioxidant[6-9] activities and as a chemo preventive agent in several cancer cells[10-14]. Structurally curcuminoids are linear diaryl heptanoids which exist in tautomeric forms as α,β unsaturated 1,3-diketo form and enol form. Curcuminoid analogues prepared by synthesis retain the α,β unsaturated 1,3-diketo moiety and their metal chelates possess remarkable biochemical activity[15-18].

In the present work, aldehydes namely 2,4-dihydroxy benzaldehyde and 2,5-dimethyl benzaldehyde were condensed with acetylacetone in

presence of B_2O_3 using tri-secondary butyl borate and n-butyl amine as the condensing agent[19]. The ligands prepared[1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(HL_1), 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (HL_2)] were complexed with Al(III), Ga(III) & In(III) to form metal chelates. The curcuminoid analogues and their metal chelates were subjected to *in vitro* cytotoxic studies using trypan blue exclusion method [20]. *In vivo* antitumour studies were conducted in DLA induced mice. The curcuminoid analogues and their metal chelates were administered intraperitoneally (i.p.) as drug into the mice and the % increase in life span was calculated and compared with standard drug [21]. The ligands and the metal complexes were also subjected to antibacterial activity against the test organisms *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis*.

2. Materials and methods

The chemicals required were obtained from Sigma Aldrich chemical suppliers and are of analar grade. Bacterial strains namely *Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis* were obtained from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India. Daltons Lymphoma Ascites (DLA) and Ehrilich Ascites Carcinoma (EAC) cells were obtained from the Adayar Cancer Research Institute, Chennai, India and propagated as transplantable tumours in Swiss albino mice by injecting a suspension of cells ($1X10^6$ cells/ml) intraperitoneally.

Swiss albino mice were obtained from the Small Animal Breeding Station (SABS), Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. They were kept under standard conditions of temperature and humidity in animal house of Amala Cancer Research Centre. All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (No.149/1999/CPCSEA).

2.1. Analytical instruments

UV spectra were recorded on a Schimadzu UV-VIS-1601 spectrophotometer. IR spectra (KBr pellets) were recorded on 8101 Schimadzu FT IR spectrophotometer. The ^1H NMR spectra were recorded on a Varian 300 NMR spectrophotometer. The FAB mass spectra were recorded on a Joel SX–102 mass spectrophotometer from CDRI, Lucknow, India. C, H, N analysis was done using Vario EL III analyzer.

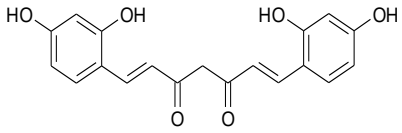
2.2. Synthesis of 1,7 – diaryl – 1,6 – heptadiene – 3,5 – diones

The curcuminoid analogues were prepared by the condensation of aldehydes (2,4-dihydroxy benzaldehyde and 2,5-dimethyl benzaldehyde) with acetylacetone boric oxide complex in ethyl acetate medium in presence of tributyl borate and n – butyl amine [19]. The product was purified by column chromatography over silica gel (60 – 120 mesh) using 4:1 (v/v) chloroform**:**acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material (Figure2.1).

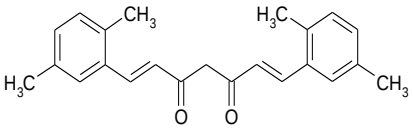
Fig.2.1. Structure of curcuminoid analogues

(HL_1) 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione (1a)

1a



1b



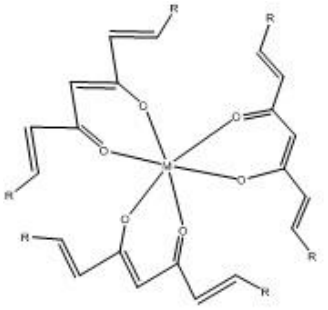
(HL_2) 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (1b)

2.3. Synthesis of metal complexes

The Al(III) complexes were prepared by adding a methanolic solution of aluminium nitrate $Al(NO_3)_3.9H_2O$ (25 ml, 0.001mol) to a solution of diketone (25 ml, 0.003 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1**:**1, methanol**:**water mixture and recrystallised from hot methanol. The Ga(III) & In(III) complexes were prepared by adding a methanolic solution of gallium(III) chloride and indium(III) acetate (25 ml, 0.001mol) respectively to a solution of diketone (25 ml, 0.003 mol) in methanol and the above procedure is repeated(Figure2.2).

Fig.2.2. Structure of metal complexes

M - Trivalent metal



2.4. Antibacterial assay (Agar well diffusion method)

Agar plates were prepared using sterile Muller-Hinton (MH) agar medium. Bacterial strains of *Escherichia Coli, Klebsiella Pneumoniae* and *Bacillus Subtilis* of 24 h culture were evenly spread into the surface of the agar plates using sterile swab sticks. Wells were cut into agar plates with sterile gel puncture. The curcuminoid analogues and their metal chelates in the concentration 5 mg/ml in DMSO were added in the cells. The pure solvent DMSO act as negative control and streptomycin (5mg/ml) served as positive control. The plates were incubated at 37°C for 24 h and observed for zones of inhibition. The antibacterial activity was measured in terms of mean diameter of the zone of inhibition in mm.

2.5. *In vitro* cytotoxicity study

In vitro cytotoxicity studies were carried out using the diketones, Al(III), Ga(III) & In(III) complexes dissolved in minimum quantity of DMSO. These compounds (as drugs) with concentrations 200, 100, 50, 20 & 10 µg/ml were dissolved in minimum quantity of DMSO. The tumour cells (DLA & EAC), aspirated from the peritoneal cavity of tumour bearing mice were washed with PBS (Phosphate Buffered Saline). Cell viability was determined by trypan blue exclusion method. Viable cells (1×10^6 cells in 0.1 ml) were added to tubes containing various concentrations of the test compounds and the volume was made up to 1 ml using PBS. Control tube contains only cell suspension. These mixtures were incubated for 3 h at 37°C. Further, cell suspension was mixed with 0.1mol of 1% trypan blue and kept for 2-3 min. and loaded on a haemocytometer. The number of stained (dead cells) and unstained (live) cells were counted and percentage cytotoxicity was evaluated by trypan blue exclusion method [20].

2.6. *In vivo* antitumour activity

Animals (male mice, 6-8 weeks old) weighing 28-30g were divided into 11 groups of 5 animals each. Viable DLA cells ($1X10^{6)}$ in 0.1ml of phosphate buffered saline (PBS) were injected into the peritoneal cavity of mice. Group1, Control: Oral administration of 0.1 ml of distilled water/animal. Group 2, Standard: Cyclophosphamide 25mg/kg body weight. Group 3-5: Ligand, 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione with concentrations 20µg/ml, 10µg/ml and 5µg/ml was given as drug. Group 6-8 & 9-11: Al(III) & In(III) metal chelates as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively. Ligand, complexes and cyclophosphamide were given by i.p. injection from the 1st day of tumour induction upto 10 days. The death pattern of animals due to tumour burden was noted and the percentage increase in life span (ILS) was calculated.

% ILS= {(T – C)/ C} X 100,

Where T and C are mean survival of treated and control mice resplectively.

3. Results

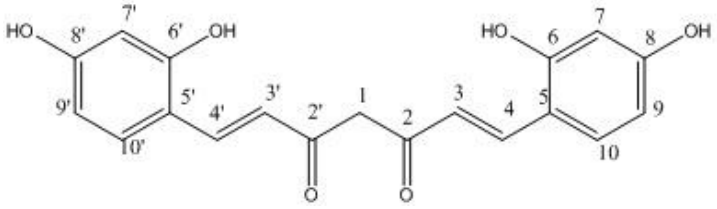
3.1. Spectral data of 1,7-diaryl-1,6-heptadiene-3,5-diones

The compounds prepared were characterized on the basis of UV, IR, ^{1}HNMR & Mass spectral data (Table3.1). The ^{13}CNMR spectral data of the two ligands were also given in Table3.2 & 3.3.

Table3.1. UV, IR, ^{1}H NMR & Mass spectral data of 1,7- diaryl heptanoids

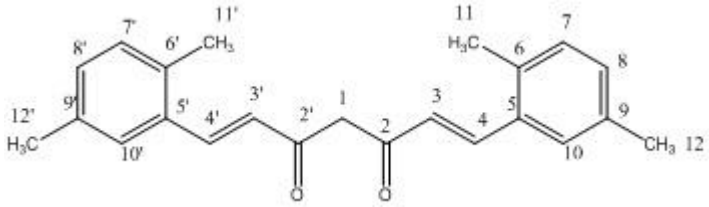
Compound	UV data λmax	IR data cm^{-1} ν(C=O)	1HNMR spectral data (δ ppm)				Mass spectral data (m/z)
			Enol	Methine	Phenyl	Phenolic	
HL_1	265, 431	1619	16.8	6.9	7.1-7.9	10.04	341,231,205,177, 163,135,122
HL_2	286, 393	1622	16.2	6.5	7.1-7.9	_	332,299,173,159, 145,138,115

Table3.2. ^{13}C NMR spectra of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione



C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'	C7,C7'	C8,C8'	C9,C9'	C10,C10'
102.4	185.5	122.45	121.46	139.54	135.65	130.32	134.35	130.2	134.82

Table3.3. ^{13}C NMR spectra of 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione



C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'
101.82	183.48	124.83	126.76	138.33	130.81
C7,C7'	C8,C8'	C9,C9'	C10,C10'	C11,C11'	C12,C12'
134.92	133.73	130.77	135.72	20.98	19.33

3.2. Spectral details of metal complexes

1,7-Diaryl heptanoids form well defined crystalline complexes with Al(III), Ga(III) and In(III) ions. Analytical and mass spectral data (Table3.4) clearly suggest a ML_3 stoichiometry for all the metal complexes.

Table3.4. Spectral data of Al(III), Ga(III) and In(III) complexes of 1,7 – diaryl heptanoids

Complex	UV spectra λmax	IR data cm-1		Mass spectral data (m/z)
		ν(C = O)	ν(M-O)	
$Al(L_1)_3$	272,430	1613	472,410	1048,937,828,707,340
$Ga(L_1)_3$	270,440	1591	463,413	1090,979,870,750,340
In(L1)3	273,443	1601	468,415	1135,1024,915,794,110
$Al(L_2)_3$	284,392	1602	460,410	1024,920,815,691,359
$Ga(L_2)_3$	289,385	1612	490,420	1065,961,856,715,332
$In(L_2)_3$	288,390	1609	490,435	1110,898,839,799,666

3.3. Antibacterial activity

The antibacterial activity of 1,7-diaryl heptanoids and their complexes were depicted in Fig.3.1 andFig.3.2. Bacterial strains of *Escherichia Coli, Klebsiella Pneumoniae* and *Bacillus Subtilis* were used against the curcuminoid analogues and their metal chelates (Al & In) in the concentration 5 mg/ml in DMSO. The results were compared to the std. drug streptomycin.

Fig.3.1. Antibacterial activity of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione and their metal complexes

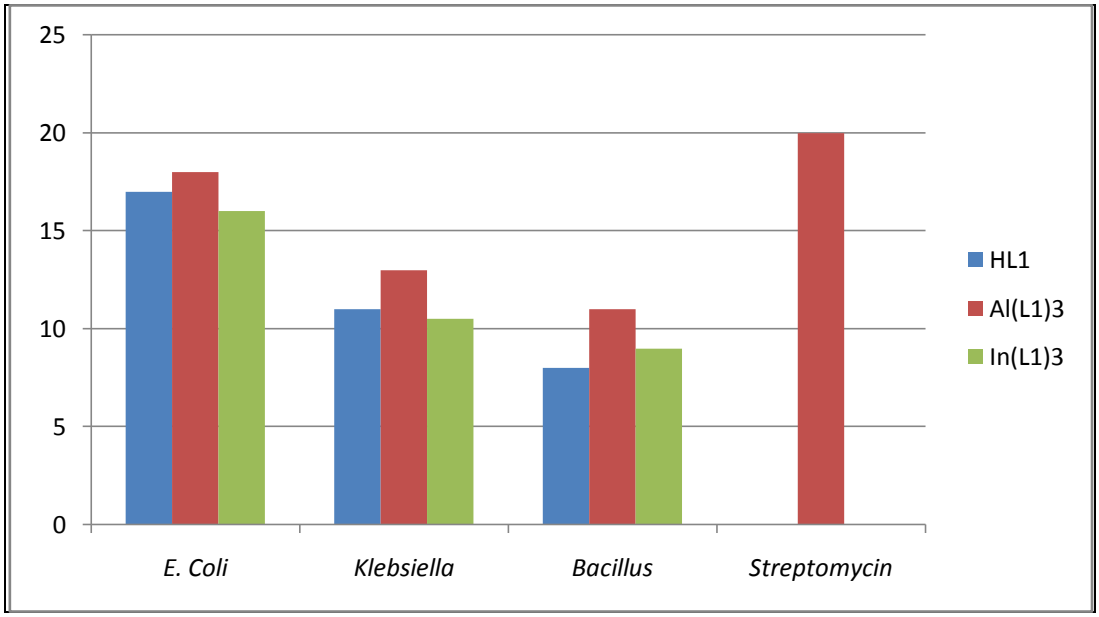
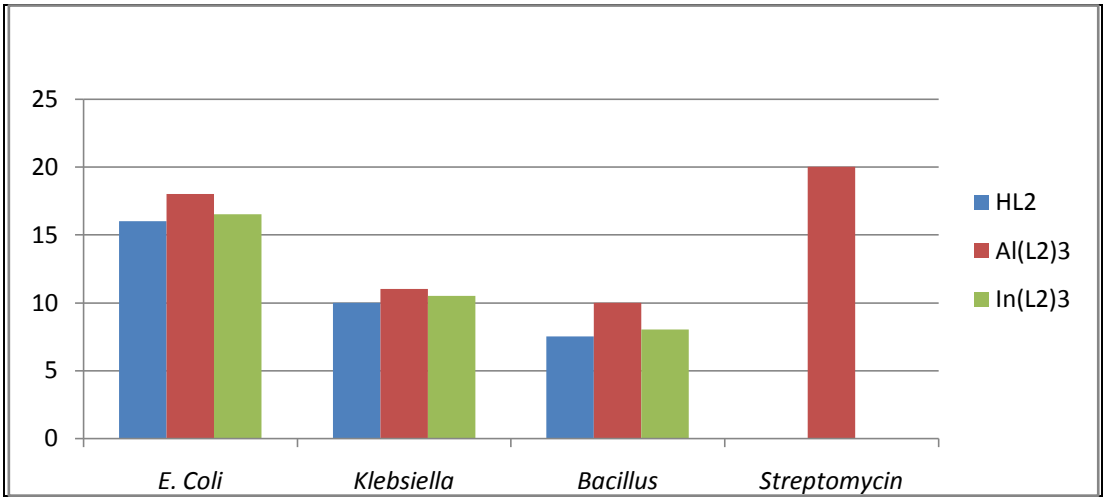


Fig.3.2. Antibacterial activity of 1,7-bis((2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione and their metal complexes



3.4. *In vitro* cytotoxicity

In vitro cytotoxicity studies were carried out using the diketones (HL_1 & HL_2), and their Al(III), Ga(III) and In(III) complexes towards EAC & DLA cells. These compounds (as drugs) with concentrations 200, 100, 50, 20 & 10 µg/ml, were dissolved in minimum quantity of DMSO. The results were given in Figures 3.3, 3.4, 3.5, and 3.6.

Fig.3.3. *In vitro* cytotoxicity of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione and their metal complexes towards EAC

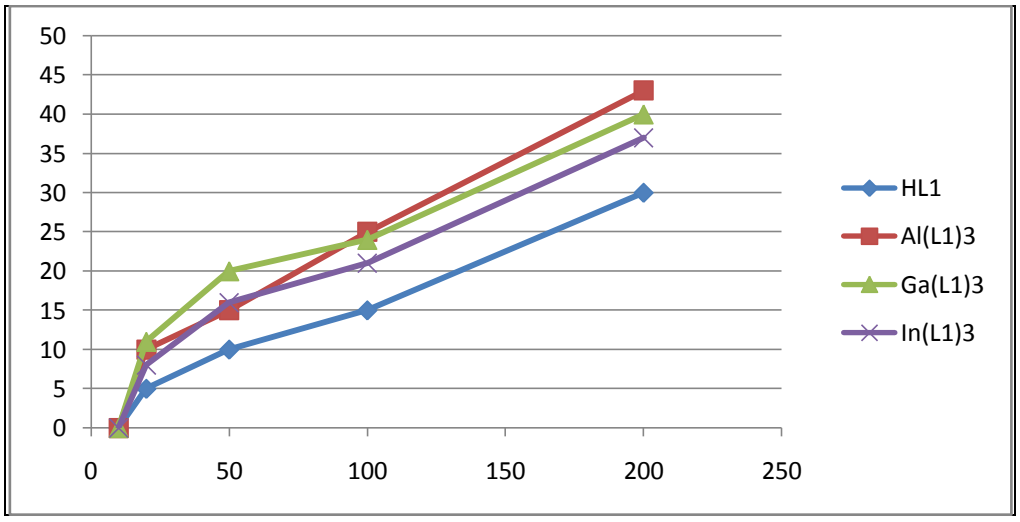


Fig.3.4. *In vitro* cytotoxicity of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione and their metal complexes towards EAC

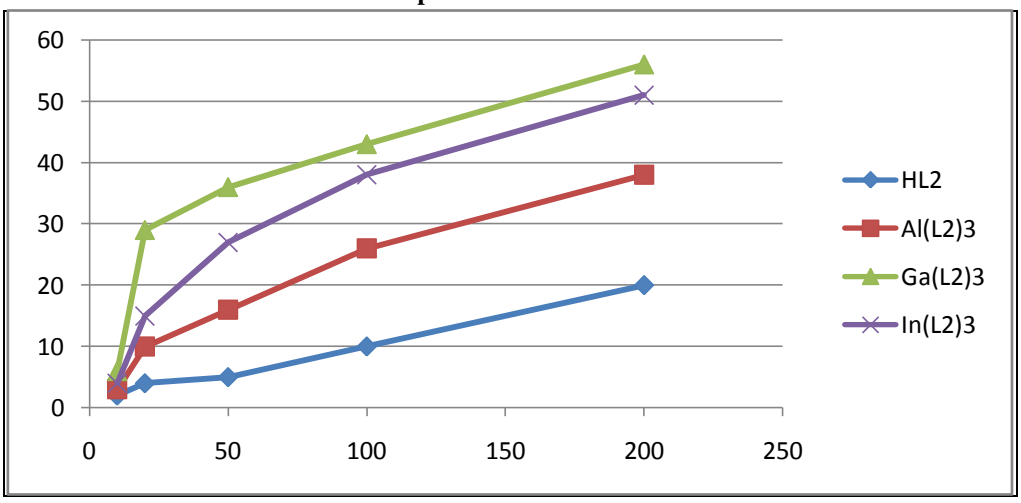


Fig.3.5. *In vitro* cytotoxicity of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione and their metal complexes towards DLA

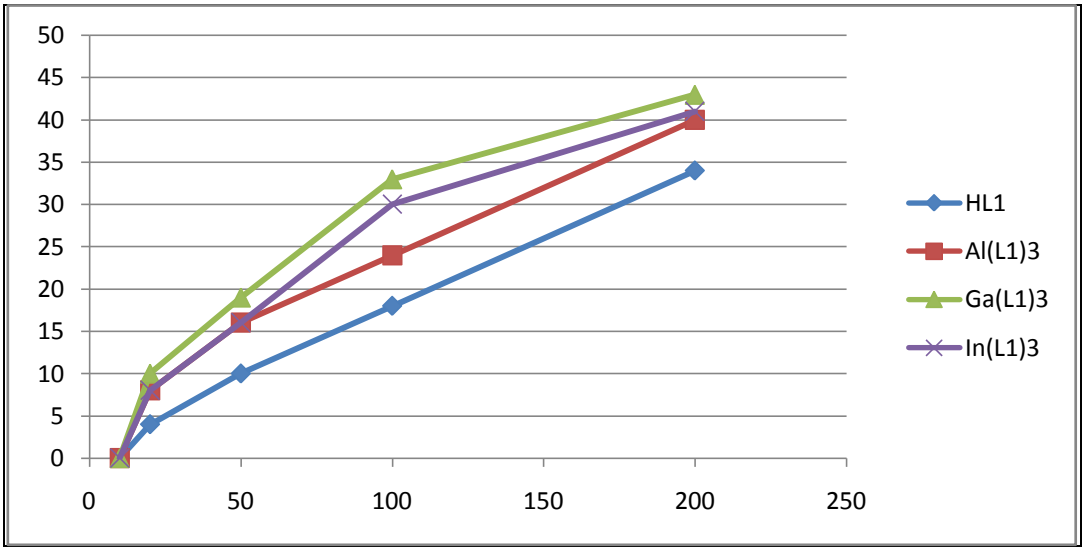
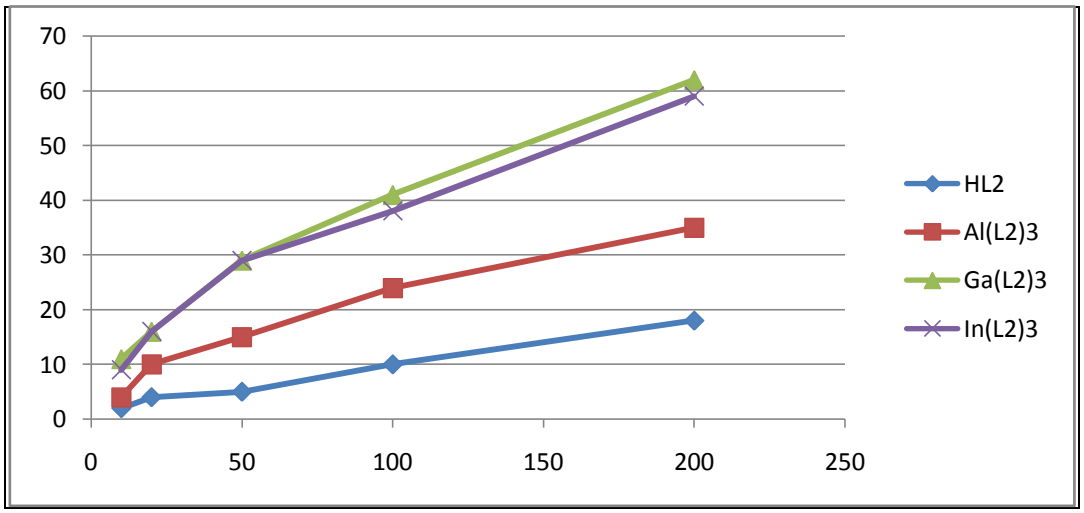


Fig.3.6. *In vitro* cytotoxicity of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione and their metal complexes towards DLA



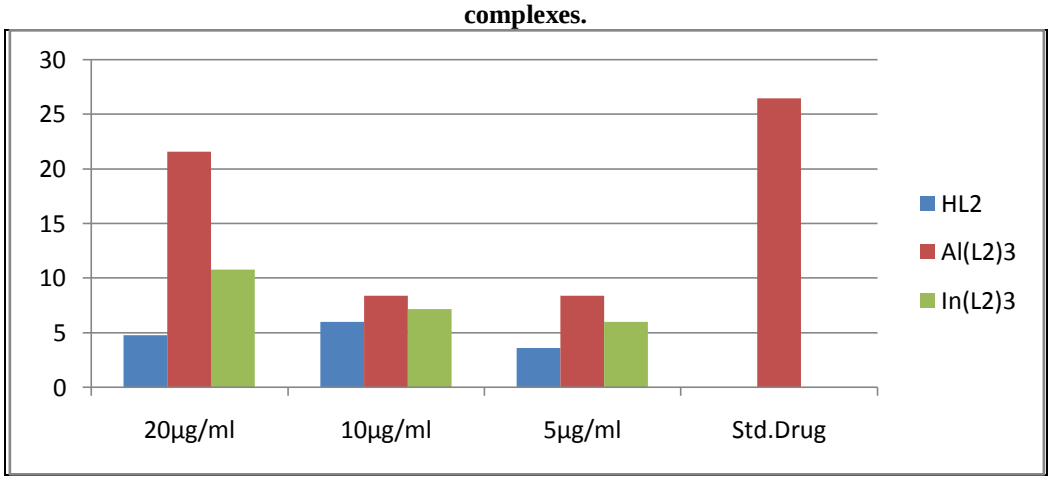
3.5. Effect of compounds on ascites tumour reduction (*in vivo)*

The ligand 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (HL_2) & its metal complexes Al(III) & In(III) were given as drug and the survival of animals are given in Table 3.5. The control group and the group with std. drug cyclophosphamide are also given in the table. The values of No. of days survived are means of five determinations $\pm SD$ (standard deviation). The increase in life span (% ILS) corresponding to drugs HL_2, $Al(L_2)_3$ and $In(L_2)_3$ with varying concentrations compared with std. drug cyclophosphamide is also given in Figure 3.7.

Table3.5. Effect of compounds on ascites tumour reduction

Animal groups	Concentration µg/ml	No.of animals with tumour	No. of days Survived	% ILS
1. Control		5/5	16.6±1.49	
2.Standard drug		5/5	21.0±5.09	26.5
3.HL_2	20	5/5	17.4±2.65	4.8
4.HL_2	10	5/5	17.6±1.85	6.02
5.HL_2	5	5/5	17.2±2.13	3.6
6.$Al(L_2)_3$	20	5/5	20.2±3.12	21.6
7.$Al(L_2)_3$	10	5/5	18±2.83	8.4
8.$Al(L_2)_3$	5	5/5	18±1.78	8.4
9.$In(L_2)_3$	20	5/5	18.4±2.15	10.8
10.$In(L_2)_3$	10	5/5	17.8±3.18	7.2
11.$In(L_2)_3$	5	5/5	17.6±1.85	6.02

Fig.3.7. The % ILS of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione and their Al and In complexes.



4. Discussion

4.1. Structural characterization of ligands

The UV spectra of the ligands in methanol show two absorption maxima corresponding to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The IR spectra of compounds show a strong band ~1620 cm^{-1} assignable to intra molecularly hydrogen bonded carbonyl function. The ^{1}HNMR spectra of the compounds show peaks due to enol, methine and alkenyl proton. Additionally compound HL_1 shows a singlet at δ ~10.04 ppm due to phenolic group. Peaks corresponding to step wise elimination of aryl groups and small fragments are present in the mass spectra. In both the cases the peaks corresponding to the molecular ion peak are very evident. The ^{13}CNMR spectra of the compounds show specific peaks at δ ~ 101 & 183ppm corresponding to C_1 and C_2 carbon atoms. The peaks corresponding to phenyl moiety is also clearly visible at δ~ 130ppm. The peak due to alkenyl moiety is present at δ~ 120ppm. In HL_2 the peaks due to two methyl groups are present at δ 20.98 & 19.33ppm respectively.

4.2.Structural characterization of metal complexes

In the IR spectra of metal chelates, the bond due to intra molecularly hydrogen bonded carbonyl function of the ligand at ~1620 cm^{-1} disappeared and instead a strong band assignable to stretching of the coordinated carbonyl moiety appeared at ~ 1600 cm^{-1}. Additional bands appear at ~ 475 cm^{-1} and at ~ 420 cm^{-1} assignable to ν (M–O) vibration. In the UV spectra, the peaks present in the ligands were retained, i.e, the two absorption maxima corresponding to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions are unaltered. The mass spectra of complexes showed relatively intense peak at m/z corresponding to ML_3 stoichiometry. The molecular ion peak and the peaks corresponding to the step wise elimination of one ligand, two ligands and small fragments including aryl groups are very clear.

4.3. Antibacterial activity of compounds

The antibacterial activity of 1,7-diaryl heptanoids (HL_1 & HL_2) and their complexes towards three bacterial strains, i.e, *Escherichia Coli*, *Klebsiella Pneumoniae* and *Bacillus Subtilis* were studied. The results show that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin. The activity of these compounds towards the bacteria *Escherichia Coli* was maximum. Comparatively lesser activity was found towards the other two bacteria. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance activity. Out of the two metals, aluminium complexes show maximum antibacterial activity

4.4. *In vitro* cytotoxicity of ligands and complexes

In vitro cytotoxicity studies were carried out using the diketones (HL_1 & HL_2), Al(III), Ga(III) & In(III) complexes. These compounds (as drugs) with concentrations 200, 100, 50, 20 & 10 µg/ml, were dissolved in minimum quantity of DMSO and were subjected to Cytotoxic studies against tumour bearing cells EAC & DLA. It was observed that almost comparable results were obtained with metal complexes of HL_1 towards EAC and a slight edge was observed for Al complexes. But in the case of HL_2 towards EAC, Ga complexes have the maximum value. The in vitro cytotoxicity of HL_2 towards EAC follows the sequence Ga > In > Al > ligand. The in vitro ytotoxicity of HL_1 & HL_2 towards DLA show almost similar trend and follows the same sequence as above. The overall results indicate that metal chelation enhance cytotoxicity of compounds considerably. The compounds with concentration 200µg/ml show maximum activity. Generally Ga & In complexes of 1,7-diharyl heptanoids show better results than that of ligands as well as Al complexes. Ga complexes of both the ligands show a high degree of activity.

4.5. *In vivo* antitumour activity

In vivo antitumour studies were carried out with different groups with the ligand 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(HL_2) and its metal complexes Al(III) and In(III) against the tumour bearing cell DLA. A control group and group with standard drug cyclophosphamide were also carried out. The animals of the tumour control group inoculated with DLA survived for a period 16.6±1.49days. Treatment with cyclophosphamide, survived for 21±5.09 days. The animals which were given the drug HL2, survived for 18±2.6 days with the concentration 20µg/ml. The maximum value of No. of days of survival was observed with Al complexes with con. 20µg/ml, i.e, 20.2 days comparable to that of 21 for std. drug. The %ILS was also calculated and the values of ligand and complexes given are comparable to that of std. drug cyclophosphamide. The increase in life span for $Al(L_2)_3$ was maximum (21.6%) with 20µg/ml con. This is also comparable to that of cyclophosphamide (26.5%).

The ongoing discussion reveals that the 1,7-diaryl heptanoids and their metal complexes possess enhanced antitumour (both *in vivo & in vitro*) activity. The metal chelation considerably enhances the cytotoxicity of these compounds. Generally Ga & In complexes of 1,7-diharyl heptanoids show better results than that of Al(III) complexes in the *in vitro* cytotoxicity studies both with EAC and DLA. The antibacterial studies of both ligand and metal complexes show enhanced activity. The Al(III) complexes show better antibacterial activity than complexes and ligands. The *in vivo* antitumour studies of Al(III) complexes show more activity than In(III) complexes and ligands. The results are very much comparable to that of std. drug cyclophosphamide.

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