IJRD International Journal of Research & Development Organisation

ISSN: 2455-6653

Journal of Applied Science

Biochemical activities of Curcuminoid analogues with Methyl substituted phenyl ring and their Transition metal chelates

Seena Thomachan[a], S.Sindhu[b] and John V D[c].

[a,b,c]Department of Chemistry, Christ College (Affiliated to University of Calicut), Irinjalakuda-680125, Kerala, India

* *Correspondence Info :*
Seena Thomachan, Research Scholar,
Department of Chemistry,
Christ College (Affiliated to University of Calicut),
Irinjalakuda- 680125, Kerala, India.
E-mail: thomachanseena@yahoo.com
Mobile Number:+919388445530

Abstract

In recent years there has been an increased enthusiasm in treating the diseases by natural products.Curcumin has found a wide application in this context.Curcumin and its analogues have been extensively studied for their biological activities including antimicrobial,anti-inflammatory,antioxidant,anticarcinogenic etc. Recently a number of compounds structurally related to curcuminoids were synthesized and their chemotherapeutic potential has been revealed. In the present study, the synthesis and characterization of two curcuminoid analogues with methyl substituted phenyl ring and their metal chelates Cu(II),Zn(II),Oxovanadium(IV)&Ni(II) are discussed here. The curcuminoid analogues namely 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1) and 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) and their metal chelates were synthesized and were characterized using UV, IR, ^{1}H NMR and mass spectral data. *In vitro* cytotoxic studies were done with ligands and metal complexes against EAC cells(Ehrilch Ascites Carcinoma) using Trypan 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(Daltons Lymphoma Ascites) in mice and compared with standard anticancer drug cyclophosphamide. The life spans of the treated animals were increased. The present investigation reveals that the Cu(II) complexes show enhanced cytotoxic activity and antibacterial activity than the curcuminoid analogues ,Zn(II),Ni(II) and Oxovanadium(IV) complex.

Keywords: 1,7–diarylheptanoids;IR; NMR; mass spectra; antitumour; cytotoxicity;antibacterial

1. INTRODUCTION

Curcumin is an important phytochemical derived from the rhizomes of turmeric (*Curcuma longa Linn*) , a member of ginger family, Zingaberaceae. In Ayurveda, turmeric has been used as medicine for various indications.All curcuminoids are components of turmeric and often referred to simply as curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3, 5-dione].Turmeric contains a variety of different curcuminoids namely curcumin,demethoxycurcumin and bisdemethoxy curcumin.Curcuminoids has been isolated from the ground rhizome part of the curcuma plant species. Curcumin has several biological effects exhibiting anti inflammatory[3,4,7,19,20],antifungal,anticarcinogenic and antioxidant activities[8,13,21,22]. It has also been studied extensively as a chemo preventive agent in several cancer cells[1,2,6,9,15,23]. Structurally curcuminoids are linear 1,7-diaryl-1,6-heptadiene-3,5-diones 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 but the aryl ring in natural curcumin is modified and here in the present study it is replaced with a methyl substituted phenyl ring .

Curcuminoids are expected to form metal complexes similar to other 1,3-diketones.They are powerful chelating agents.[10] Scientific research spanning over more than four decades have confirmed the diverse pharmacological effects of curcumin and is still attracting the researchers from, all over the world. Inorganic chemists have used its metal chelating abilities through the β diketo group to form new structural entities with modified biochemical activities. Complexation with transition metals has attracted much interest over the past years as one of the useful requirement to treat deadly diseases.Curcuminoids and their metal chelates possess

remarkable biochemical activity[5,11,12].HereCu(II),Zn(II),Ni(II) andOxovanadium(IV)complexes of curcuminoid analogues are synthesized and characterized.

In the present study, aldehydes namely 2-methyl benzaldehyde and 2,5-dimethyl benzaldehyde were condensed with acetylacetone in presence of B2O3 using tri-secondary butyl borate and n-butylamine as the condensing agent[17]. The ligands prepared [Fig 1.1-1.2] were complexed with Cu(II),Zn(II)Ni(II)and VO(II) to form metal chelates[Fig1.3] . The curcuminoid analogues and their metal chelates were subjected to *in vitro* cytotoxic studies using trypan blue exclusion method[14].*In vivo* antitumour studies were conducted in DLA induced mice[10]. 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. The ligands and the metal complexes were also subjected to antibacterial activity against the test organisms *Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis*[16,18].

The structure of ligands and metal complex is given below

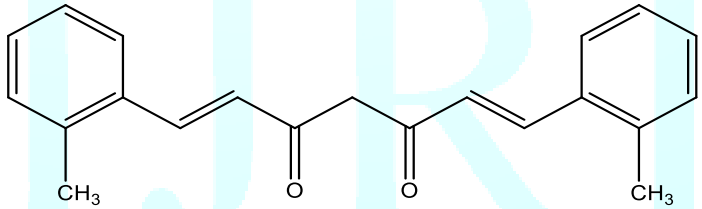

Fig 1.1 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1)

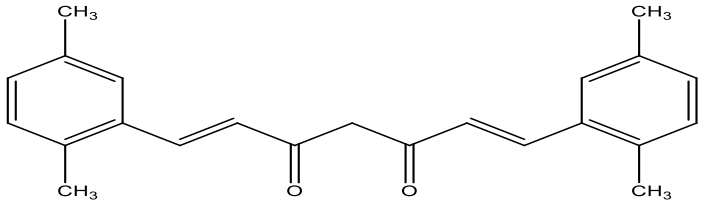

Fig 1.2 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)

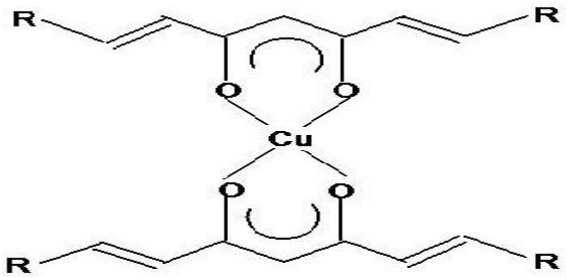

Fig 1.3 Cu(II)chelate of ligands

2. MATERIALS AND METHODS

The chemicals required were obtained from Sigma Aldrich chemical suppliers and are of analar grade. 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. Bacterial strains namely *Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis* were obtained from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India.

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 FTIR spectrophotometer. The ^{1}H 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.

2.2. *Synthesis of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1) and 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)*

The curcuminoid analogues were prepared by the condensation of aldehydes(2-methyl benzaldehyde and 2,5-dimethyl benzaldehyde)with acetyl acetone-boric oxide complex in ethyl acetate medium in presence of tributyl borate and n-butyl amine. 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.

2.3. *Synthesis of metal complexes*.

The Cu(II) complexes were prepared by adding a methanolic solution of copper(II) acetate (25 ml, 0.001mol) to a solution of curcuminoid analogue (25 ml, 0.002 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 Cu(II) complexes were synthesized and characterized.

The Zn(II) complexes were prepared by adding a methanolic solution of zinc(II)acetate(25ml,.001mol)to a solution of curcuminoid analogue(25ml,.002mol)in methanol and refluxed gently for 2h. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

The Oxovanadium(IV) complexes were prepared by adding a methanolic solution of vanadyl sulphate(25ml,.001mol)to a solution of curcuminoid analogue(25ml,.002mol)in methanol and refluxed gently for 2h. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

The Ni(II) complexes were prepared by adding a methanolic solution of nickel acetate(25ml,.001mol)to a solution of curcuminoid analogue(25ml,.002mol)in methanol and refluxed gently for 2h. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

2.4. In vitro cytotoxicity studies

In vitro cytotoxicity studies were carried out using the diketone and Cu(II) ,Zn(II),Ni(II)and oxovanadium(IV)complexes dissolved in minimum quantity of DMSO. The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed with PBS (Phosphate Buffered Saline) and centrifuged for 15min. at 1500 rpm. 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 1ml using PBS. Control tube contains only cell suspension. These mixtures were incubated for 3h at 37^{o}C. Further, cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 min. and loaded on a haemocytometer. The number of stained (dead) and unstained (live) cells were counted and percentage cytotoxicity was evaluated by trypan blue exclusion method .

% Cytotoxicity=(No:of dead cells/No:of dead cells+No:of live cells)x100

2.5. *Antibacterial assay (Agar well diffusion method)*

Agar plates were prepared using sterile Muller-Hinton (MH) agar medium.Bacterial strains of *Escherichia Coli, KlebsiellaPneumoniae* and *Bacillus Subtilis* of 24 hr 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.

2.6. ***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-no test compound is injected. Group 2, Standard:standard anticancerdrug Cyclophosphamide 25mg/kg body weight is injected. Group 3-5: Ligand**1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)**with concentrations 20µg/ml, 10µg/ml and 5µg/ml was given as drug. Group 6-8 Cu(II) metal chelate of ligand L2 as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively.Group 9-11: VO(II) chelate of ligand L2 was given as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively.The ligands ,metal complexes and cyclophosphamide were given by i.p. injection from the 1stday 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 respectively.]

3. RESULTS

3.1. ***Structural characterization of synthesized ligands*****:**

The synthesized curcuminoid analogues namely1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1) and 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) were synthesized and characterized by UV, IR, ^{1}HNMR and Mass spectral data **(Table 3.1).**

Table 3.1: UV, IR, ^{1}H NMR & Mass Spectral data of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1)& 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)

Compound	UV data Λ max (nm)	IR data cm-1 ν (C=O)	^{1}H NMR spectral dataChemical shift (ppm)					Mass spectral data (m/z)
			Enol	Methine	Functional group (methyl)	Phenyl	Alkenyl	

L1	298, 376	1631	16.025	5.838	2.345	7.071-7.415	6.529-7.954	305,220, 194,163, 149,122, 105
L2	281, 393	1622	15.941	5.835	2.463	7.199-7.269	6.536-7.97	332,227, 202,173, 159,127, 115

3.2. *Structural characterization of metal complexes with Cu(II),Zn(II),Ni(II),&VO(II)ions*

Both the ligands L1andL2 form well defined crystalline complexes with Cu(II),Zn(II),Ni(II) and VO(II) ions. Analytical and mass spectral data of synthesized metal complexes are given in (**Table 3.2).** The approximate formulae of the metal complexes has been found to be as expected ie two ligands are coordinated with the metal ion.(ML2).

Table 3.2: Spectral data of Cu(II) Zn(II),Ni(II)&Oxovanadium(IV)complexes of ligands

Complex	**UV spectra Λ max (nm)**	**IR data (cm-1)**		**Mass spectral data (m/z)**
		V (C=O)	**V (M-O)**	
Cu(L1)2	279,398	1598	464,415	671,489,369,307,154,145,121
Zn(L1)2	282,399	1592	465,433	669,487,367,305,182,154
Ni(L1)2	280,397	1595	465,426	664,482,362,300,179,121
VO(L1)2	281,393	1585	456,428	672,490,370,308,188,154
Cu(L2)2	284,394	1608	460,430	726,516,395,306,185,121
Zn(L2)2	289,397	1610	470,420	728,518,397,308,187,

Ni(L2)2	286,395	1602	465,435	721,511,390,301,180,121
VO(L2)2	290,398	1615	479,438	729,519,398,309,188,121

3.3. *In vitro cytotoxicity*

The results of *in vitro* cytotoxicity of ligands namely 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1)& 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) and their complexes Cu(II)),Zn(II),Ni(II)and VO(II) towards EAC cells are given in Table**. 3.3**. The diketones and their metal complexes are given as drug in concentrations 200µg/ml, 100µg/ml, 50µg/ml, 20µg/ml & 10µg/ml. The number of stained and unstained cancer cells were counted and evaluated as % cell death.

Table 3.3 shows % cytotoxicity towards EAC cells for curcuminoid analogues and Cu(II),Zn(II),Ni(II),VO(II) chelates

Concentration	L1	$Cu(L1)_2$	Zn(L1)2	VO(L1)2	Ni(L1)2	L2	Cu(L2)2	Zn(L2)2	VO(L2)2	Ni(L2)2
200µg	31	70	47	58	50	20	80	34	43	38
100 µg	23	50	32	53	35	10	66	20	40	21
50µg	18	29	20	35	25	5	47	8	20	13
20µg	10	15	12	14	12	4	35	5	7	6
10µg	2	8	6	7	7	2	15	4	5	5

3.4. *Antibacterial activity*

The results of the antibacterial activity of **of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1)& 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)** and their complexes with Cu(II)), Zn(II) ,VO(II)are given in **Table 3.4.**For comparison the diameter of zone of inhibition in mm given by the std.drug has also been included in the table.

Table 3.4: Antibacterial activity of ligands and their metal complexes

Bacteria	Diameter of zone of inhibition in mm.							
	L1	Cu(L1)2	Zn(L1)2	VO(L1)2	L2	Cu(L2)2	Zn(L2)2	VO(L2)2
E Coli	11	16	14	12	16	19	18	16.5
Klebsiella	10	14	12	11	11	18	16	12
Bacillus	8.5	13	10	9	9.5	14	15	11
Streptomy-cin (std)	20	20	20	20	20	20	20	20

3.5. *Effect of compounds on ascites tumour reduction*

The ligand **1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)** & its metal complexes(Cu&VO(II)) were given as drug and the days of survival of animals were found.The results are given in **Table 3.5**.The No.of days survived by the control 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 ±SD (standard deviation). The increase in life span corresponding to drugs L2 and Cu & Vanadyl complex with varying concentrations is also given..

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

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	26.4±3.6	59.03
3L2	20	5/5	18.0±2.60	8.0
4.L2	10	5/5	17.6±2.72	6.02
5.L2	5	5/5	17.4±2.87	4.8
6.Cu(L2)$_2$	20	5/5	25.0±2.63	50.6
7.Cu(L2)$_2$	10	5/5	23.2±2.65	39.75
8.Cu(L2)$_2$	5	5/5	21.4±2.71	28.9
9.VO(L2)$_2$	20	5/5	20.8±2.15	25.30
10. VO(L2)$_2$	10	5/5	19.2±2.99	15.66
11. VO(L2)$_2$	5	5/5	18.4±1.86	10.84

4. DISCUSSION

4.1 Characterization of methyl substituted analogues of 1,7-diaryl-1,6-heptadiene-3,5-diones

The synthesized methyl substituted derivatives of 1,7-diaryl heptanoids were characterized by various analytical techniques **(Table 3.1)**. The UV spectra of the compound in methanol show two absorption maxima corresponding to n $\rightarrow$ π^* and $\pi \rightarrow \pi^*$ transitions The UV spectra of the compound L1 in methanol show two absorption maxima at 376nm &298nm respectively due to the $n \rightarrow \pi *$ and $\pi \rightarrow \pi *$ transitions. The value at 270-300nm are due to $\pi \rightarrow \pi *$ transition and at 360-460nm are due to $n \rightarrow \pi *$ transitions based on earlier reports.The presence of α,β unsaturation increases the wavelength of carbonyl absorption maxima.The high energy band at 298nm is due to $\pi \rightarrow \pi *$ transition of the fully conjugated system.The UV spectra of the compound L2 in methanol show two absorption maxima at 393nm &281nm respectively due to the $n \rightarrow \pi *$ and $\pi \rightarrow \pi *$ transitions .

IR spectra of L1 and L2 are characterized by the presence of strong bands at 1631 cm^{-1} and 1622 cm^{-1} respectively due to the enolised conjugated C=O group. The stretching frequency of acetyl carbonyl group and carbonyl stretching frequency of aroyl group are at ~ 1710 cm^{-1} and at ~ 1660 cm^{-1} respectively. The C=O

frequency decreases due to hydrogen bonding and increased conjugation. There is no other band in the region 1600-1800 cm^{-1} which is assignable due to free or bound C=O group.This shows that the compound exist in the intramolecularly hydrogen bonded enolic form.In the spectra, the intramolecular hydrogen bonded enolic group shows a broad band in the region 2550-3600 cm^{-1}. There are a number of medium intensity vibrations observed in the region 1550-1600 cm^{-1} due to various stretching vibrations of the phenyl group, alkenyl & chelate ring.The band in the region 979 cm^{-1} and 974 cm^{-1} is assigned to the trans CH=CH vibration.

The ^{1}H NMR spectra of methyl substituted 1,7-diaryl heptanoids also supports the enolic structure of the compound.The peaks corresponding to enolic, methine, alkenyl, methyl and phenyl groups can be observed in the spectrum.**.** Ligands **L1 & L2** displayed a one proton singlet at ~ 16ppm assignable to strong intramolecularly hydrogen bonded enolic proton . Another one proton singlet at ~ 5.8ppm can be assigned to the strong intramolecularly hydrogen bonded methine proton. The aryl protons show signals in the region 7.1 – 7.5ppm and the alkenyl protons show signals in the region of 6.5 – 8.0ppm. The methyl group on aryl ring in L1 showed a signal at 2.345 where the two methyl groups on aryl ring of L2 are present at ~ 2.5ppm.

The most important application of mass spectra is in the determination of molecular weight of compounds.The molecular ion peaks as well as fragment ion peaks are observed in the spectra.The mass spectra also gives idea about the various fragmentation modes of the substance. The mass spectra of both compounds showed intense molecular ion peaks. Elimination of important groups like CH2,C2H2,C2H2O,CH2=C=O from the molecule gives different fragments.Important peaks appeared in the spectra of compounds can be conveniently accounted by the fragmentation pattern . The (M+1) ion of L1 is observed at 305 and the M+ ion of L2 is observed at 332.The remaining important peaks are that due to the fragment ions.

4.2 Characterization of metal complexes

Analytical and mass spectral data (**Table 3.2**) clearly suggests a stochiometry ML2 for complexes. The UV absorption bands of the ligands were almost unaffected by complexation with metal ions. The spectra of complexes closely resembles the spectra of respective ligands. So there is no much change in the structure due to complex formation. There is a bathochromic shift of absorption maxima to longer wavelength which indicate the involvement of the carbonyl moiety in chelate formation.

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 ~ 420 cm^{-1}assignable to ν (M–O) vibrations. The absence of a strong band in the region 1650-1800 cm^{-1} is one

characteristic feature of the metal complex.But the peak due to intramolecularly hydrogen bonded carbonyl group which is present at ~ 1630 cm^{-1} disappeared and a new band appeared at ~ 1595 cm^{-1}. The new band can be assigned to the metal coordinated carbonyl group .The replacement of enolic proton by a metal ion is also evident from the absence of the broad band in the region of 2600 -3500 cm^{-1} present in the ligand.

The main feature of NMR spectra of metal complex is the absence of singlet signal at δ ~ 16ppm which was due to the enolic proton present in the ligands.This indicates the replacement of enolic proton by metal atom in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation. There is a slight shift of methine signals to the downfield of the spectra. Thus the spectra of ligand and complexes are much similar except those of enolic proton.

In their mass spectra,all the complexes showed relatively intense peaks at m/z corresponding to ML_2 stochiometry,where M is metal and L is ligand.Mass spectral fragments are another important tool in elucidating the structure of metal complexes. It was found that some fragments rearrange to form stable cyclic species . The mass spectral analysis shows that stepwise removal of aryl groups is a characteristic feature of all the complexes. In all the cases $[ML_2]+$ ion is the most intense peak. Smaller molecules like O, OH, CH etc. are also eliminated.Peaks due to $[ML_2]+$,L+ and fragments of L+ are also detected in the spectrum.

4.*3 In vitro cytotoxicity*

Invitrocytotoxicity studies towards EAC cells revealed that both ligands and complexes exhibited greater %cell death at higher concentrations ie 200µg/ml.As concentration of drug compound increases the % cell death increases.Comparing the ligands,L1 with one methyl ring was found to be more cytotoxic than L2 with two methyl rings.All the metal complexes showed significant increase in % cell death than the ligands.Among the metal complexes the activity followed the order Cu(II)>VO(II)>Ni(II) > Zn(II). It is also noted that metal chelation enhances cytotoxicity of compounds considerably. The copper complexes of methyl substituted 1,7-diarylheptanoids show better results than that of ligands in almost all concentrations. Cu(II)chelate of ligand 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)showed maximum cytotoxicity among the metal complexes.The complex was very effective in increasing %cell death and produced 80%cell death.The activity of Cu(II) complex was nearly four times that of the ligand.

4.4 *Antibacterial activity*

The data in Table 3.4 clearly reveal that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin. In all the cases, metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance activity. Among the

ligands 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) is active against all bacterial strains.It exhibited greater activity against E.Coli bacteria.The Cu(II) and Zn(II) complexes were quite effective against bacterial strains.The VO(II) complex showed activity comparable with the ligands.The Cu(II) complex of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) produced a zone of inhibition of 19mm which is very much comparable with 20 mm zone of inhibition produced by the std.drug.

4.5. *Effect of compounds on ascites tumour reduction* (*in vivo*)(Table3.5)

The animals of the tumour control group inoculated with DLA survived for a period 16.6±1.49 days. The animals treated with cyclophosphamide, survived for 26.4±3.6 days. The animals which were given the drug 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) showed 8% increase in its life span with the concentration 20µg/ml.The Cu(II) complexes showed %ILS of 50.6,39.75 and 28.9 at different concentrations namely 20,10,5µg/ml respectively and for VO(II) complexes the %ILS was 25.30,15.66 and 10.84 respectively. The increase in life span for $Cu(L2)_2$ was maximum (50.6%) with 20µg/ml concentration and it is nearly six times that of ligand. This is also comparable to that of cyclophosphamide,the std.drug which produced %ILS of 59.03.The VO(II) complex showed a % increase in life span which is about four times that of ligand. The studies conducted reveal that metal complexes especially Cu(II) complex is very effective in reducing tumour development in mice.

CONCLUSION

The ligands1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L1)& 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) and their complexes Cu(II)),Zn(II),Ni(II)and VO(II) were synthesized and characterized by various spectral techniques.The ongoing discussion reveals that the methyl substituted derivatives of 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. Also it is found that Cu(II) complex of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) is the most active compound in *in-vitro* cytotoxicity study with EAC cells . The antibacterial studies clearly show that both ligand and metal complexes possess significant antibacterial activity. The Cu(II)complexes show better antibacterial activity than Zn(II) and VO(II) complexes and ligands.The Cu(II)complex of ligand 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2)showed maximum antibacterial activity. The ligand 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L2) was found to be not very effective in reducing ascites tumour in mice when compared with its Cu(II) complex.The Cu(II)complex possessed maximum activity which is comparable with a standard

anticancerous drug and produced a considerable increase in the life span of tumour bearing mice. The results are very much comparable to that of std. drug cyclophosphamide.

Acknowledgement

The authors would like to thank Dr.Girija Kuttan,Professor, Amala Cancer Research Institute,Thrissur,India for the antitumour studies and Dept. of Biotechnology, St. Joseph's College, Irinjalakuda, Thrissur, India for antibacterial studies.

References

[1] Al-Hujaily,E.M., Mohamed,A.G., Al-Sharif, I.(2011)PAC- a novel curcumin analogue, has anti-breast cancer properties with higher efficiency on ER-negative cells. *Breast Cancer Res Treat.* **128**,97–107

[2]Azuine, M.A, Bhide, V.(1992) Chemo preventive effect of turmeric against stomach and skin tumours induced by chemical carcinogens in Swiss mice. *Nutrition Cancer.* 17(1), 77-83.

[3]Chainani-Wu, N(2003). Safety and Anti-Inflammatory Activity of Curcumin. *J. Altern. Complement. Med.* 9,161-8

[4]Chandra, D., Gupta, S.S.(1972) Anti inflammatory and anti arthritic activity of volatile oil. *Indian Journal of Medical Research.* ,60, 138-140.

[5]Clare, M.J., Hydes, D.C. In: Sigel H (Ed.)(1979). Metal ions in biological systems, metal complexes as anticancer drugs. *Marcel Decker, New York: p. 1-62.*

[6] Fadda,A., Badria ,F., El-Attar,K.(2010) Synthesis and evaluation of curcumin analogues as cytotoxic agents.*Medicinal Chemistry Research.* **19**,413–*30.*

[7]Holt, P.R., Katz, S., Kirshoff, R.(2005) Curcumin therapy in inflammatory bowel disease: a pilot study. *Dig. Dis. Sci.*, 50.

[8]Iqbal, M., Sharma, S.D., Okazaki, Y., Fujisawa, M., Okada, S.(2003) Dietary supplementation of curcumin enhances antioxidant and Phase II metabolizing enzymes in ddy male mice: possible role in protection against chemical carcinogenesis and toxicity. *Pharmacol.Toxicol*, 1, 92.

[9]Kostova, D., Albena ,T., Paul, T.(1999) Relation of structure of curcumin analogues to their potencies as inducers ofphase-2 detoxification enzymes. *Carcinogenesis*. 20**,** 911-919.

[10]Krishnankutty, K., John, V.D.(2003) Synthesis, characterization and antitumour studies of metal chelates of some synthetic curcuminoids. *Synhesis and Reactivity in Inorganic Metal-Organic Chemistry.* 33, 343-358

[11]Krishnankutty, K., John ,V.D., Kuttan, G.(2002) Antitumour studies of metal chelates of synthetic curcuminoids. *Journal of Experimental and Clinical Cancer Research. 21, 219-224*

[12]Krishnankutty, K., Venugopalan, P.(1998) Metal chelates of curcuminoids. *Synthesis and Reactivity in Inorganic Metal-Organic Chemistry.* 28,1313-1325.

[13]Kuo, M.L., Huang, T.S., Lin, J.K.(1996)Curcumin, an antioxidant and anti-tumour promoter. *Biochim.Biophys.Acta*. 2, 1317.

[14]Kuttan, G., Vasudevan, D.M., Kuttan, R(1988). Isolation and identification of a tumour reducing component from mistletoe extract. *Cancer Letters. 41, 307-315*

[15]Leu, T.H., Maa, M.C.(2000) The Molecular mechanism for the Antitumorigenic effect of curcumin *Curr. Med. Chem. Anti-canc Agents, 2.*

[16]Othman, A., Hamed,Noha Mehdawi,Adhan Abu Taha.(2013)Synthesis and Antibacterial activity of Novel curcumin derivatives containing Hetero cyclic Moiety.*Iran J Pharm Res Winter*

[17]Pabon, H.J.J.(1964) A synthesis of curcumin and related compounds. *Recueil des TravauxChimiques des Pays-Bas. 83, 237-240.*

[18]Raman,N,.Muthuraj,V,.Ravichandran,S.,Kulandaisamy,A.(2003)Synthesis,Characterisation & electrochemical behavior of Cu(II), Co(II),Ni(II) and Zn(II) complexes derived from acetyl acetone and their antimicrobial activity.*Proc.Indian Acad Sci(Chem.Sci),115(3),161-167*

[19]Sharma, R.A., Gescher, A.J., Steward, W.P.(2005) Curcumin: The story so far. *Eur. J. Cancer*, 41.

[20]Shishodia, S., Sethi, G., Aggarwal, B.B.(2005) Curcumin: getting back to the roots. *Ann N Y Acad. Sci* , 1056.

[21]Sreejayan, N., Rao, M.N.A.(1994) Curcuminoids as potent inhibitors of lipid peroxidation. *J. Pharm. Pharmacol.* , 46.

[22]Subramanian, M., Sreejayan, N., Rao, M.N.A, Devasagayam,T.D.A,Singh, B.B(1994). Diminution of singlet oxygen-inducedDNA-Damage by *curcumin and related antioxidants. Mutat Res*, 311.

[23] Wilken,R., Veena,M.S., Wang,M.B., Srivatsan,E.S.(2011) Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma, *Mole. Can.* **10**, 1-19.