The Possible Role of Reactive Centre's of Curcumin in Deciding its Biological Activity

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Abstract: The aim of the study was to investigate the role of keto-enol centre of curcumin in deciding its ROS quenching efficiency, toxicity and DNA binding ability. Curcumin has three major contributing centers for free radical reaction, namely phenol, enol and conjugated diene. The activity of metal complex of curcumin and curcumin was compared to evaluate the effect of keto-enol reactive center towards the biological and antioxidant activity. The metal complex of curcumin exhibited ROS quenching efficiency comparable to that of curcumin emphasising importance of phenolic centre. Morphological studies with H9c2 cells revealed insignificant levels of alterations in 48 h of treatment with curcumin and its metal complexes. The comparable cytotoxicity value of curcumin and its metal complexes point to the insignificance of keto-enol centre in deciding its activity. The complexes of curcumin showed better binding capability in the order of 10^5 in comparison to our previously reported curcumin binding of the order 10^3. The change in the curcumin scaffold at keto-enol center by metal retained its ROS quenching efficiency, exhibiting comparable cytotoxicity to that of curcumin at the same time improving the binding capability to DNA emphasizing the importance of phenolic centre in deciding its activity and complexation with metal has modest effect its antioxidant property.

Key words: Curcumin, metal complex, H9c2 cells, ROS, cytotoxicity, DNA binding.

1. Introduction

Over the past 20 years, there has been a growing interest in plant-derived polyphenols with respect to human health. All the compounds in plants have been selected by evolution to have biological importance and that plants also generate novel compounds with extreme stereospecificity. Consequently, 50% of the anticancer therapeutic agents used in modern medicine are derived from plants. Curcumin, the vibrant yellow spice derived from the rhizome of the plant, *Curcuma longa* is an interesting polyphenolic compound with a wide range of pharmacological property. It is one of the most potent and thoroughly researched dietary phytochemical in cancer management and has been used to treat a range of health problems of India and China [1]. Clinical trials suggest its viability as an alternative to non-steroidal anti-inflammatory drugs or COX-2 inhibitors, the potential colorectal cancer chemo preventive agent [2]. Various mechanisms of action like G1/S arrest and apoptosis induction other than the mitotic block in different tumour cell lines are responsible for the anticancer activity of curcumin [3, 4]. A multitude of molecular targets were identified for curcumin like cell cycle proteins, cell surface adhesion

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protein, transcription factors, enzymes and cytokines [5, 6]. Further, curcumin is the first polyphenolic compound found to bind in the minor groove of nucleic acids [7].

Curcumin chelate to electropositive metals via 1,3 diketo group and may act as a scavenger of toxic metal to reduce the metal load in the body. The role of curcumin as a neuro protector against metal deposition in brain has been investigated and hence regarded as anti-Alzheimer's [8]. The strong antioxidant property exhibited by the compound and its strong interaction with many other biological species enhances its use as multi-targeting agent [9]. Curcumin is reported to form 1:1 and 2:1 type complexes with metals like copper, iron and other transition metals [10-12]. The binding of curcumin in brain cells to metals like iron and copper reduces the concentration of these toxic metals in brain, hence suggested as a multipotent agent for combating oxidative stress and Alzheimer disease (AD) [13]. A few biological studies of metal complexes of curcumin and its derivatives have been reported. Copper complexes of curcumin mimic SOD [10], manganese complex was shown to have neuroprotective activity [12]. The Vanadium, Gallium and Indium complexes showed reduced cytotoxic potential and hence expected to have medicinal applications [14].

The low bioavailability and poor stability of curcumin in aqueous medium especially at high pH limits its therapeutic applications though it has evident anti-cancer activity [1]. Consequently many curcumin analogues with superior efficacy and stability than natural curcumin were synthesized, nevertheless to ensure the safety of such congeners, extensive and elaborate long-term toxicity studies would be necessary. Intuitively, minor alterations without affecting the active centre of the parent curcumin stand a better chance of retaining the safety features as compared to more complex alterations. Our group recently reported the equivalent efficacy for Knoevenagel condensates of curcumin for ROS quenching efficiency *in vitro* selenite-induced cataract

model analyzed using lens organ culture method [15]. Knoevenagel condensate exists only as diketo form, as condensation prevent the keto-enol tautomerism of diketo moiety of curcumin. Hence it was concluded that the enol moiety is not a definite requisite for its antioxidant property [16]. To establish these findings and to minimize the involvement of groups introduced during Knoevenagel condensation, the study was extend to metal complex of curcumin in which the enol/diketo centre ligands to the metal ion. The cytotoxicity and DNA binding studies of metal complex of curcumin were also included to evaluate their therapeutic applications, since they have minor alteration from curcumin scaffold and have high stability in aqueous system [17]. The present group has reported that curcumin is a groove binder with a binding constant 1.1×10^3 M^{-1} [18]. The DNA binding study of the metal may reveal the influence of the metal in deciding its binding ability.

2. Materials and Methods

2.1 Materials

Commercial sample of curcumin was purchased from Kancor Ingredients Ltd, Angamaly, Kerala, India. The metal salts, Cupric chloride [$CuCl_2 \cdot 2H_2O$], Magnesium chloride [$MgCl_2 \cdot 6H_2O$], Cobalt chloride [$CoCl_2 \cdot 6H_2O$], Manganous chloride [$MnCl_2 \cdot 4H_2O$] were purchased from S.D. Fine Chem. Ltd., Mumbai, India. Nickel chloride [$NiCl_2 \cdot 6H_2O$] was from Universal Laboratories Private Ltd. Mumbai, India. The analytical grade piperidine and solvents were purchased from Merck, India and S.D. Fine Chem. Ltd, India. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and 2′,7′dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma, St. Louis, MO, USA. Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS) and tris(hydroxymethyl)aminomethane hydrochloride, (Tris-HCl) were purchased from Himedia Pvt. Ltd. Mumbai, India. Herring sperm DNA was procured

from Sisco Research Laboratories Pvt. Ltd., India.

2.2 Methods

2.2.1 Separation of Curcumin from Commercial Sample and the Preparation and Characterization of Its Metal Complexes

Curcumin was separated from the commercial curcumin, a mixture of three closely related curcuminoid's, that is curcumin (77%), demethoxy curcumin (DMC) (18%) and bisdemethoxy curcumin (BDMC) (3%), by column chromatography (silica gel 60-120 mesh) [19]. The 1:1 metal complexes were prepared (Scheme 1) using curcumin by the procedure reported earlier [20]. ^{1}H NMR spectra were recorded in a Bruker Advance DRX 300 FT-NMR spectrometer with TMS as the internal standard. CHN analysis was done on Elementar model Vario EL III CHN analyser. FT-IR spectra were recorded by KBr pellet method with JASCO-8000 Fourier Transform Infrared Spectrophotometer in the range 400-4,000 cm^{-1}. EDXRF set up, consisting of Radioisotope Cd^{109} induced X-ray fluorescence spectrometer and Si (Li) detector of resolution 170 eV at 5.9 KeV. Mn X-rays was used to estimate the metal content in the complex. The molar conductivity of the curcumin complexes in dimethylsulphoxide (DMSO) (10^{-3} M) were measured at room temperature using direct reading conductivity meter (Systronic conductivity bridge type 305). The cell imaging was done with BD Pathway™ Bioimager system, USA. UV-Vis spectra for DNA binding were recorded on a Shimadzu UV-160 spectrophotometer. Electrochemical measurements (cyclic voltammetry and differential pulse voltammetry) were made in Tris–HCl/NaCl buffer (50 mM Tris–HCl/1 mM NaCl buffer, pH 7.1) using dimethylsulphoxide (DMSO) solution of the complexes of curcumin using a BAS CV-50W electrochemical analyzer. The three-electrode cell comprised with a reference Ag/AgCl, auxiliary Pt and the working glassy carbon electrode (GC electrode with surface area of 0.07 cm^2) was used for the measurement. The surface of GC electrode was cleaned with alumina powder before every measurement. n-Bu_4NClO_4 (TBAP) was used as the supporting electrolyte for CV measurement of complexes in DMSO . All the experimental solutions were purged with N_2 for 15 min prior to each set of experiments. Circular dichroism spectra of DNA were recorded on a JASCO J-810 spectropolarimeter in the region between 220 and 320 nm in Tris-HCl buffer (pH 7.1 at 25 °C) using quartz cuvette of 5 mm optical path length.
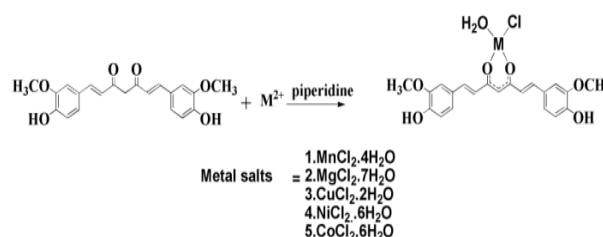
Scheme 1   Synthesis of metal complex of curcumin.
  Curcumin, piperidine, methanol, stirring at room temperature for 4 h.

2.2.2 Cardiomyocyte Model in H9c2 Cells

H9c2 cells derived from rat embryonic cardiomyocytes were obtained from National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) supplemented with 10% foetal bovine serum (FBS), 100 U penicillin/mL, and 100 μg streptomycin/mL and cultured in 5% CO_2 at 37 °C. Cells were passaged regularly and subcultured to 80% confluence before the experiments. Morphological evaluation, MTT assay and generation of reactive oxygen species (ROS) was studied in this cell line.

2.2.3 In vitro Cytotoxicity Assay

2.2.3.1 Morphological Analysis

H9c2 cells in the exponential growth phase were plated at 5×10^4 cells in 24-well plate. Curcumin and metal complexes of curcumin (5 μM) in DMSO was added to wells such that the total concentration of DMSO is less than 0.1% in the culture medium. After 48 h, cells were analyzed for morphological alterations under the phase-contrast microscope (Nikon Eclipse TS100, Japan) at 40 × magnifications.

2.2.3.2 MTT Assay

Cells in the exponential growth phase were suspended in the medium (DMEM). A total of 5×10^4 cells per well were seeded in 24-well plate. After 48 h of incubation at 37 °C, cells were subjected to treatments with various compounds at 1, 5, 10, 15 and 20 µM concentrations for 24 h. 350 µL of MTT solution (5 mg MTT mL^{-1} DMEM) was added to each well and incubated for 3-4 h at 37 °C. Viable cells with active metabolism convert MTT into a purple coloured formazan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into formazan. The formazan crystals formed were dissolved in DMSO. The plate was read after 45 min of incubation at room temperature in a microplate reader (Biotek Synergy 4, USA) at 570 nm.

2.2.4 Generation of ROS

Intracellular ROS content was determined by oxidative conversion of cell-permeable DCFH-DA to fluorescent 2′,7′ dichlorofluorescein (DCF). H9c2 cells were seeded in 96-well plate at a density of 5000 cells per well. The experimental group consist of (1) control cells; (2) cells for H_2O_2 treatment (positive control); (3) cells pre-treated with curcumin (5 µM); (4) cells pre-treated with Cu-curcumin (5 µM); (5) cells pre-treated with Mn-curcumin (5 µM); (6) cells pre-treated with Ni-curcumin (5 µM) and (7) cells treated with Co-curcumin (5 µM) for a period of 24 h. Experimental groups (2-7) were treated with H_2O_2 for 15 min, prior to staining. DCFH-DA solution in serum free medium was added at a concentration of 10 µM and co-incubated with the various groups at 37 °C for 20 min. After three washes to remove excess stain that may interfere the image background DCF fluorescence was measured by fluorimetry (530 nm) in multiwell plate reader (Biotek Synergy 4, US) and fluorescent imaging was done (BD Pathway™ Bioimager system, USA) to detect the difference in the intensity of fluorescence emitted.

2.2.5 DNA Binding Study

Samples Stock solution of DNA (~10^{-3} M) was prepared by dissolving 50 mg of herring sperm DNA in 25 mL of Tris-HCl buffer (50 mM NaCl + 5 mM Tris-HCl mixture; pH 7.1) and sonicated for 25 cycles of 30s each with 1 min intervals. The concentration of DNA was determined spectrophotometrically by measuring its absorbance at 260 nm (molar extinction coefficient is 6600 M^{-1}·cm^{-1} at 260 nm after 1:100 dilutions [21]. Stock solutions were stored at 4 °C and used over not more than 4 days. The fixed concentration of complex in DMSO: Tris-HCl buffer (1:9) ratio of mixture was titrated with different concentration of DNA and its variation was recorded in UV spectrophotometer and CV. In DNA binding study using the CD, titration was done with fixed concentration of DNA and variable concentration of metal complexes.

2.2.6 Statistical Analysis.

All statistical calculations were carried out using Microsoft excel 2010 software program. The values were expressed as the mean of Standard deviation with a minimum 3 trials (n = 3). The data were statistically analyzed using one-way analysis of variance (ANOVA), and significant difference of means was determined using Duncan's multiple range tests at the level of $p < 0.05$.

3. Results and Discussion

3.1 Characterization of Curcumin Complexes

Curcumin separated from commercial sample by column chromatography [19] was characterized by NMR and used for the complex preparation [20]. Analytical data (Table 1) suggest that all complexes were mononuclear and have a metal to curcumin ratio 1:1. Non-electrolytic nature of formed complexes were evidenced by the molar conductance value in DMSO, which is in agreement with the earlier report of 1:1 complexes of curcumin [17, 10] and have been assigned molecular formula [M(II)Cur·H_2O·Cl].

All complexes were brown in colour with λ_{max} values for UV absorption ranging from 437-443 nm which was in agreement with the suggestions of Barik

Table 1 Analytical data of metal complexes of curcumin. Values in bracket are theoretically calculated values [C-carbon, H-hydrogen, M-metal and Cl-chlorine]. Molar conductance values are expressed in (Ohm^{1}·cm^{2}·mol^{1}). Absorbance at (λ_{max}), for UV-visible spectral data.

Sample	C%	H%	M%	Cl%	Mol con	Abs
Mn-cur	53.88	4.70	11.30	7.85	6	442
	(53.01)	(4.45)	(11.55)	(7.45)		
Mg-cur	56.76	4.56	5.90	8.21	17	437
	(56.66)	(4.76)	(5.46)	(7.96)		
Cu-cur	52.71	4.25	13.18	7.66	20	439
	(2.07)	(4.37)	(13.12)	(7.32)		
Ni-cur	52.34	4.50	12.50	7.98	22	443
	(52.60)	(4.41)	(12.24)	(7.39)		
Co-cur	52.14	4.73	12.50	7.89	20	438
	(52.07)	(4.51)	(12.28)	(7.99)		

Table 2 FTIR band's of curcumin and its metal complexes and their assignments.

| Sample	ν(OH) (cm^{-1})	ν(C=O) (cm^{-1})	ν(C=C) (cm^{-1})	ν(C-Ophen) (cm^{-1})	ν(OCH$_3$) (cm^{-1})
Curcumin	3,440	1,623	1,500	1,271	1,029
Mn-Cur	3,433	1,579	1,500	1,287	1,015
Mg-Cur	3,428	1,598	1,500	1,277	1,023
Cu-Cur	3,429	1,606	1,500	1,276	1,019
Ni-Cur	3,422	1,619	1,500	1,270	1,031
Co-Cur	3,434	1,579	1,500	1,279	1,027

et al. [10] for the 1:1 complexes. For 1:2 complexes a UV absorption in the range of 370 nm was expected. The synthesis of only 1:1 complexes were attempted to retain the number of phenolic group as two, similar to the parent curcumin in order to establish the relative importance of phenolic and enolic centers to the antioxidant activity of curcumin. The IR spectral data (Table 2) of complexes showed O-H band of phenol at 3,440 cm^{-1} similar to curcumin indicative of non-involvement of phenolic O-H group in complex formation. The strong C=O stretching frequency at 1,623 cm^{-1} was shifted to lower wave length region in all metal complexes which confirm chelation of ionic enol form of curcumin with metals [22].

3.2 In Vitro Cytotoxicity Assay

Cytotoxicity assay was done to determine the potentiality of curcumin and its metal complexes to act as drug. H9c2 cells derived from rat embryonic cardiomyocytes were selected for the toxicity studies were the oxidative stress is relatively high compared to other tissues [23]. Curcumin has been shown to possess a broad range of pharmacological properties, including protective effects against oxidative stress by virtue of its strong antioxidant capacity and decrease tumor necrosis factor-alpha levels in the livers of rats [24]. However, the biological activities of metal complexes of curcumin are less studied. The morphological analysis of cells at 48 h of treatment with curcumin and its transition metal complexes showed a normal spindle shaped morphology at 5 μM concentration in Fig. 1 indicating the non toxic nature of curcumin and its metal complexes. The cell viability assay by the MTT method showed a decrease in absorbance due to foramzan with increase in concentration of curcumin and metal complexes of curcumin indicating the cell viability decreases with the concentration of the added samples By comparing with the untreated samples a plot of % toxicity with the added concentrations of the various samples are drawn and depicted in Fig. 2.
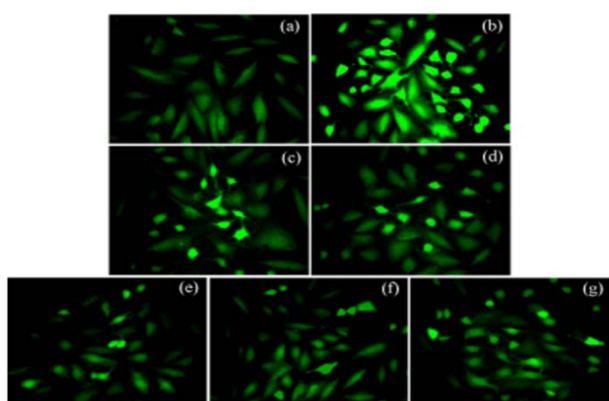
Curcumin and its metal complex [Cu(II), Mn(II), Ni(II) and Co(II)] caused reduction in cell viability in a dose-dependent manner when compared to untreated cells. The toxicity of the complexes was in the order of Cu(II) > Mn(II) > Cur > Co(II) > Ni(II) at 20 μM concentration. The result obtained was in agreement with report on Zn(II) complexes of curcumin as an ancillary ligand showing promising and selective anticancer properties and cytotoxic activity in the
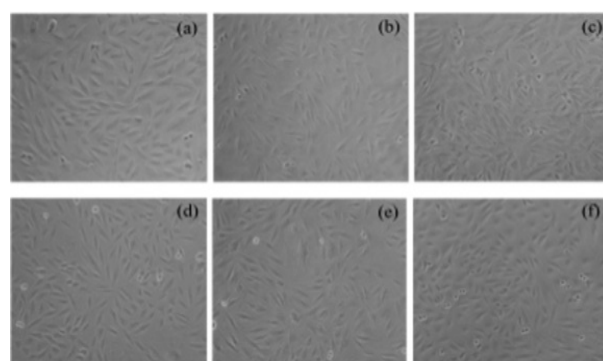
Fig. 1 Morphological features of H9c2 cells treated with curcumin and its metal complexes (5 μM) (a) Control (b) Curcumin (c) Cu-Cur (d) Mn-Cur (e) Ni-Cur (f) Co-Cur.
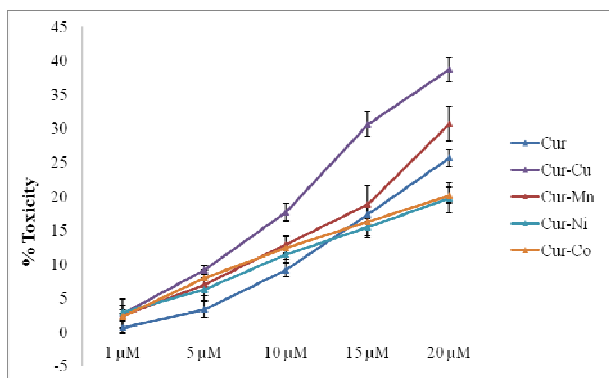
Fig. 2 MTT cell viability assay of curcumin and its metal complexes on H9c2 cell lines. Values are means, with standard deviations represented by vertical bars (n = 3).

Fig. 3 ROS quenching abilitiy of curcumin and its metal complexes (5 μM each) (a) control cells; (b) cells for H_2O_2 treatment (positive control); (c) cells pre-treated with curcumin; (d) cells pre-treated with Cu-curcumin; (e) cells pre-treated with Mn-curcumin; (f) cells pre-treated with Ni-curcumin and (g) cells treated with Co-curcumin for a period of 24 h. Experimental groups(b-g) were treated with H_2O_2 for 15 min, prior to staining.

12-37 μM dose range [25]. Whereas Quercetin and its metabolite, a phenolic antioxidant are previously reported to have shown no change in cell viability with the increase in concentration from 1-30 μM [26].

3.3 Generation of Reactive Oxygen Species (ROS)

The fluorescence imaging data Fig. 3 showed that curcumin and its complexes of Mn(II), Cu(II), Ni(II) and Co(II) reduced ROS generation in H_2O_2 treated cardiac myocytes significantly at 5 μM concentration. Fig. 3a shows slight fluorescence due to normal ROS generation in untreated cells and Fig. 3b shows very high DCF fluorescence due to the stress induced by

H_2O_2. The intensity of fluorescence was decreased in the H_2O_2 groups that were subjected to pre-treatment with curcumin/its metal complexes Figs. 3c-3g for 24 h.

The metal complexes in which the enol centre was blocked shows activity comparable to curcumin in the cell lines, emphasizing that phenolic group is the major centre for antioxidant activity. The spectro-fluorimetric analysis, Fig. 4 also supported the data obtained by fluorescent imaging. The 5 μM curcumin was able to bring down the level of ROS in H_2O_2 treated cells to that of control. The activity of curcumin and its complexes in cardiac myocytes were comparable. Among the samples taken curcumin showed the highest activity. On comparing the activity among the complexes, the Co(II) showed the highest reducing power and Cu(II) the least.

3.4 DNA-Binding Studies

3.4.1 Electronic Absorption Titration

Electronic absorption spectra of curcumin complexes in Tris-HCl buffer was characterized by an intense ligand centered transition in the visible region at 360 nm, 424 nm, 428 nm and 404 nm for Mn(II), Co(II), Mg(II) and Cu(II) complex of curcumin respectively. In the UV spectral data obtained Fig. 5
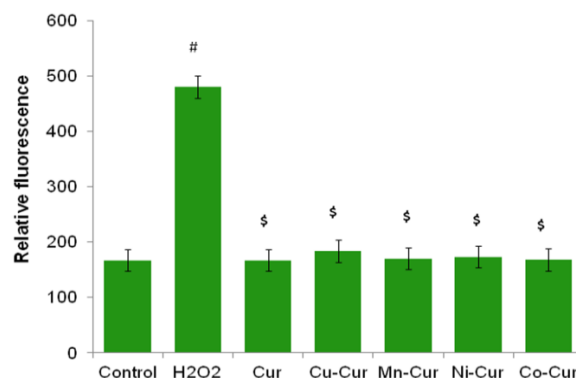
Fig. 4 Fluorescence quenching property relative to reactive oxygen species of curcumin and its Cu(II), Mn(II), Ni(II) and Co(II)complex. Values are means, with standard deviations represented by vertical bars (n = 3). "#" Mean value was significantly different from the control cells (P ≤ 0·05). "$" Mean values were significantly different from the H_2O_2 treated cells (P ≤ 0·05).
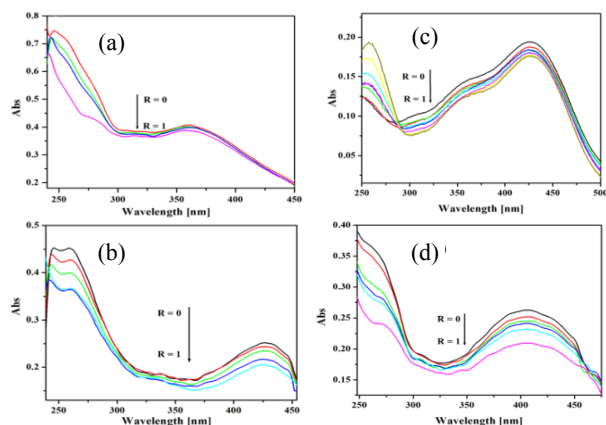
Fig. 5   UV-vis spectra of curcumin complex (a) Mn-Cur (b) Co-Cur (c) Mg-Cur (d) Cu-Cur in absence (R = 0) and presence of DNA (R = 0.1 to 1).

using fixed amount of each metal complexes of curcumin in the absence (R = 0) and with increasing concentration (R = 0.1 to 1) of HS-DNA, all the complexes exhibited hypochromism due to the interaction between electronic states of complex with DNA bases [27] with no change in band position. R is the ratio of [DNA]/[Complex].

The intrinsic binding constant K_b which illustrates the binding strength of the curcumin complexes with DNA was calculated using the Eq (1) from the spectral titration data by monitoring the changes in absorption at corresponding λ_{max} with increasing HS-DNA concentration [28]. Where, [DNA] is the concentration of DNA in base pair, ε_a is the apparent extinction coefficient for each addition of DNA to the complex (A_{abs}/[Complex]), ε_f extinction coefficient for free metal complex and ε_b extinction coefficient for metal complex in the fully bound form. A plot of [DNA]/(ε_a-ε_f) versus [DNA] gives binding constant K_b as the ratio of slope by intercept. The magnitude of K_b value gives the extent of binding. The binding constant values obtained for the curcumin complexes are in Table 3. The values were comparable to that of complexes of macrocyclic tetraaza diacetyl curcumin ligand [29] with Cu(II), Co(II) and Mn(II) as metals.

$$\frac{|DNA|}{\varepsilon_a - \varepsilon_f} = \frac{|DNA|}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \qquad (1)$$

3.4.2 Cyclic Voltammetric Studies

The voltammetric studies of DNA binding were done with two strong binders Cu(II) and Mg(II) complexes. The redox behaviour Figs. 6a and 6b studied with the fixed concentration of complexes in the absence and presence of DNA at a room temperature within the potential range of 1,000 to -400 mV at the scan rate of 100 mVs⁻¹. A decrease in peak currents (both the Ipc and Ipa) and the Epc and Epa (Table 4) with increasing concentrations of DNA was observed for both complexes. The decrease of peak current implied the formation of a new association complex and attributed to the slow diffusion of complexes bound to the large, slowly diffusing DNA molecule [30]. Among the three kinds of binding

Table 3   The Binding Constant, K_b (M^{-1}) for curcumin complexes.

| Sl.No	Compound	Binding constant K_b (M^{-1})
		Curcumin complex
1	Mn-Cur	1.9×10^4
2	Co-Cur	2.2×10^4
3	Mg-Cur	2.3×10^5
4	Cu-Cur	3.5×10^5
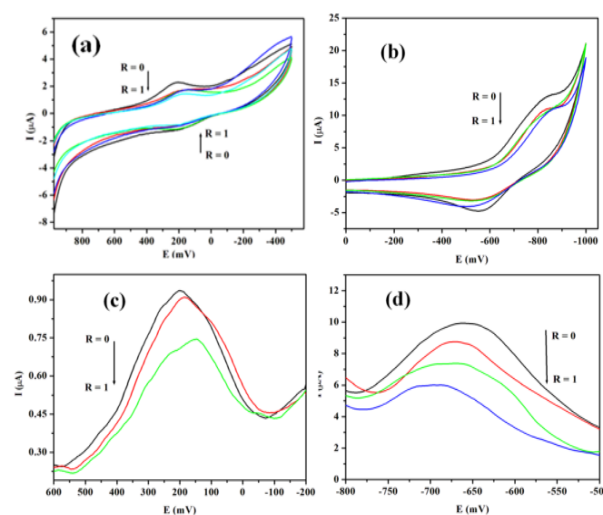
Fig. 6   Cyclic voltammogram (R = 0, 0.1, 0.4, 0.7 & 1) (a) Cu-curcumin complex (1.5 × 10⁻⁵ M) (b) Mg-curcumin complex in Tris-HCl buffer of pH 7.1 and differential pulse voltammogram of (c) Cu-curcumin complex (1.5 × 10⁻⁵ M) in the absence (R = 0) and presence (R = 0.4 and 1) of increasing amount of DNA (d) Mg-curcumin complex (1.5 × 10⁻⁵ M) in the absence (R = 0) and presence (R = 0.4, 0.8 and 1) of increasing amount of DNA.

Table 4 Voltammetric behavior of curcumin complexes in tris HCl buffer pH 7.1 in the absence and presence of DNA.

Sample	R	Epc (mv)	Epa (mv)	ΔE_p (mV)	$E_{1/2}$ (mV)
Cu-Cur	0	189	287	98	238
	0.4	154	264	110	209
	0.6	148	247	99	198
	1	138	245	107	192
Mg-Cur	0	-649	-554	-95	-602
	0.4	-658	-557	-101	-608
	0.6	-662	-559	-103	-611
	1	-678	-562	-116	-620
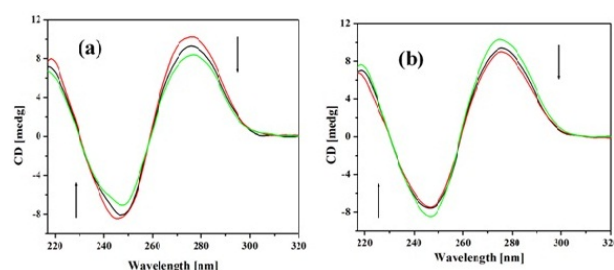
Fig. 7 CD spectra of HS-DNA (1×10^{-5} M) in tris-HCl buffer of pH 7.1 (a) in the absence (R = 0) and presence (R = 0.4 &1) of Cu-curcumin (b) in the absence (R = 0) and presence (R = 0.4 & 0.8) of Mg-curcumin.

Table 5 CD parameter for DNA-complex interaction of curcumin complex of copper and manganese.

Compound	1/R	Positive band		Negative band	
		λ_{max} (nm)	CD (mdeg)	λ_{max} (nm)	CD (mdeg)
Cu-Cur	0	275	10.2888	245	-8.5786
	0.4	275	9.4205	247	-8.4927
	1.0	277	8.6200	248	-7.4920
Mg-Cur	0	275	10.4141	245	-8.5218
	0.4	275	9.6608	247	-7.8223
	1.0	278	9.2118	248	-7.8134

modes for small molecules to DNA, the shift of Epc to a lower value is indicative of interaction mode of electrostatic or groove binding as reported by Bard [30]. The Differential pulse voltammogram (DPV); as in Figs. 6c and 6d also certify the changes found in CV experiments.

3.4.3 Circular Dichroism Studies

CD spectral variations of Herring sperm DNA (HS-DNA) were recorded by the respective addition of the curcumin complexes of copper and magnesium to the DNA. Each sample solution was scanned in the range of 220-320 nm. A CD spectrum was generated;

Figs. 7a and 7b represent the average of three scans from which the buffer background had been subtracted. The concentration of DNA was 10^{-5} M. The change in intensity of DNA band (Table 5) suggests a conformational change in DNA which is relative to the mode of binding of complex to DNA. The CD spectra showed slight perturbation of the bands with decrease in intensity in both positive and negative bands and zero cross over at 258 nm suggest the stacking mode and disturbance in the orientation of base pairs of DNA. With increasing concentration of curcumin complexes of Mg(II) and Cu(II), the peaks at 275 nm and 245 nm showed a positive shift to 1-3 nm without any change in the zero-cross over at 258 nm which indicate a groove binding mode [31]. The binding of the complexes induces certain conformational changes in DNA, that is from B-like to a more A-like structure [32]. The simple groove binding and electrostatic interaction of small molecules show less or no perturbation on the base-stacking and helicity bands, while for the classical intercalator like methylene blue enhances the intensities of both the bands stabilizing the right-handed B conformation of DNA [33].

4. Conclusions

The metal complexes of curcumin with Mn(II), Mg(II), Cu(II), Ni(II) and Co(II) was found to reduce the ROS generation in H_2O_2 treated cardiac myocytes significantly at 5 μM concentration. The study of ROS quenching ability with metal complexes along with our previously published result on antioxidant activity of the Knoevenagel condensate of curcumin with DPPH establishes the minimal involvement of keto-enol moiety of curcumin as the antioxidant centre and hold up the phenolic -OH as the prime centre for the antioxidant activity. There were no morphological alterations of H9c2 cells after 48h of treatment with curcumin and its metal complexes [Cu(II), Mn(II), Ni(II) and Co(II)] but MTT assay showed a reduction in cell viability in a dose-dependent manner. The toxicity of the complexes were comparable to that of

curcumin and was in the order of Cu(II) > Mn(II) > Cur > Co(II) > Ni(II) at 20 μM concentration. The comparable cytotoxicity values of metal complexes to curcumin signify the low involvement of keto-enol centre in deciding its activity. Curcumin complexes [Cu(II), Mn(II), Mg(II) and Co(II)] had been subjected to DNA binding studies using various techniques like UV-Vis, CV and CD spectra. The electronic spectral data exhibit a binding constants in the order of Mn(II) < Co(II) < Mg(II) < Cu(II). All the DNA-curcumin complexes showed better binding ability than our previous study with DNA-curcumin. The CD peaks at 275 and 245 nm of DNA with addition of Mn(II) and Cu(II) complex shift to 2-3 nm without any change in the zero-cross over at 258 nm. All the spectral and electrochemical investigations indicated grove binding of curcumin complex with Herring sperm.

Acknowledgments

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