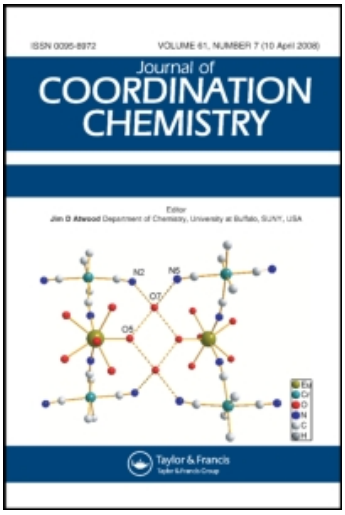

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Metal-chelating properties of carvedilol: an antihypertensive drug with antioxidant activity

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Metal-chelating properties of carvedilol: an antihypertensive drug with antioxidant activity

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Carvedilol (CarvH) (1-[carbazolyl-(4)-oxyl]-3-[2-methoxyphenoxyethyl)-amino]-2-propanol, an antihypertensive agent with β-blocker function, has been shown to act as an antioxidant. The antioxidative properties can be correlated with metal chelating ability of the drug. Iron(III), zinc(II), and copper(II) complexes of carvedilol were synthesized and characterized with respect to their structural and spectroscopic properties. Metal interaction involves O and N donors from the aliphatic moiety of carvedilol. NMR studies allowed us to obtain the structural information on metal coordination and to suggest that the physiological concentration of carvedilol and free metal ions may be enough for a protective effect by metal chelation.

Keywords: Carvedilol; Iron(III); Zinc(II); Cu(II); Chelating; Antioxidant drug

1. Introduction

Carvedilol, 1-[carbazolyl-(4)-oxyl]-3-[2-methoxyphenoxyethyl)-amino]-2-propanol, is an antihypertensive drug with multiple action [1], combining selective β-blockade and α_1 adrenoceptor blockade with an antioxidative effect *in vitro* and *in vivo*. In fact, carvedilol is a potent antioxidant, with 10-fold greater activity than vitamin E, important for its therapeutic pharmacological use [2–4].

Oxidative stress plays a critical role in biological processes such as amyotrophic lateral sclerosis, rheumatoid arthritis, aging, carcinogenesis, inflammation, and neurological diseases [5, 6]. Metal accumulation in tissues is associated with the tissue damage. For example, iron, zinc, and copper accumulation in the brain has been associated with Parkinson's, Alzheimer's, and prion diseases [7–9], while in others, as in Friedrich ataxia, excessive mitochondrial iron accumulation occurs particularly, besides in the brain, also in cardiac tissue [10]. Such accumulated metal ions can act as a catalyst in the Fenton reaction with the formation of dangerous reactive oxygen radical species.

It has been previously reported that carvedilol can sequester ferric ions in the lipid environment, whereas other β-blocker drugs are inactive. Even though antioxidative properties of carvedilol have been correlated with its coordination ability, no definitive

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information about the coordination properties of this drug towards different metals is available. For example, while one group of researchers excluded the formation of complexes with Fe(III) [11], other groups reported Fe(III) complexation by carvedilol, though no definitive conclusions about coordination sites and donors have been reported [12–14].

We previously reported the coordination ability of carvedilol towards copper(II) ions and the X-ray crystallographic structure of a copper complex has been resolved [15].

In this article, we report about the coordination ability of carvedilol toward Fe(III), Zn(II), and Cu(II) using different ligand-to-metal molar ratios. 1-D and 2-D NMR, EPR, UV-Vis reflectance spectroscopic techniques, and susceptibility measurements have been used to characterize the metal complexes. NMR spectroscopy has been used to mimic the physiological condition. In fact, though the levels of Fe(III), Zn(II), and Cu(II) are raised in several disorders, free metal ion concentrations are usually small in normal tissue and plasma.

As NMR measurements have been performed in sub-stoichiometric conditions, a possible shortcoming of this approach is represented by the eventual occurrence of complexes other than 1 : 1 or 2 : 1. For that reason, we also isolated solid compounds from the interaction of the drug with iron, zinc, and copper at different metal-to-ligand molar ratios.

2. Experimental

2.1. *Analytical and physical measurements*

2.1.1. Preparation of $[Fe(Carv)Cl_2]\cdot 1/2CHCl_3\cdot 2H_2O$ by 1 : 1 ligand : metal molar ratio. To a solution of carvedilol, CarvH (200 mg, 4.92×10^{-4} mol) in $CHCl_3$ (4 mL), a solution (30 mL) of $FeCl_3\cdot 6H_2O$ (138 mg; 4.92×10^{-4} mol) in the same solvent was added. The reaction mixture was stirred at 40°C and a small quantity of yellow powder was separated after few minutes. From this solution, orange crystals that separated after 2 days were filtered and air-dried, m.p. with decomposition was 75–88°C. Satisfactory elemental analysis for $C_{24.5}H_{29.5}N_2Fe_{3.5}O_6$ was obtained. Anal. Calcd (%): C, 46.25; H, 4.00; N, 4.00; Fe, 8.90; H_2O, 5.73. Found (%): C, 46.64; H, 4.35; N, 4.61; Fe, 8 (from atomic absorption determination); H_2O (from thermogravimetric measurement), 6.

2.1.2. Preparation of $[Fe(Carv)_2Cl]\cdot 4H_2O$ by 3 : 1 ligand : metal molar ratio. To a solution of carvedilol, CarvH (200 mg, 4.92×10^{-4} mol) in $CHCl_3$ (4 mL), a solution (30 mL) of $FeCl_3\cdot 6H_2O$ (138 mg; 1.64×10^{-4} mol) in the same solvent was added. The reaction mixture was stirred at 40°C and a yellow powder separated after few minutes was filtered and air-dried, m.p. with decomposition was 88–95°C. Satisfactory elemental analysis for $C_{48}H_{58}N_4O_{12}FeCl$ was obtained. Anal. Calcd (%): C, 59.0; H, 6.15; N, 5.74; Fe, 5.72; H_2O, 7.37. Found (%): C, 58.64; H, 5.91; N, 5.83; Fe, 6 (from atomic absorption determination); H_2O (from thermogravimetric measurement), 7.

2.1.3. Preparation of $[Cu(Carv)_2]\cdot 2H_2O$ by 8:1 ligand:metal molar ratio. To a solution of carvedilol, CarvH (200 mg, 4.92×10^{-4} mol) in MeOH (10 mL), a solution (2 mL) of $CuCl_2 \cdot 2H_2O$ (10.5 mg; 6.15×10^{-5} mol) in the same solvent was added. The reaction mixture was stirred at room temperature for few minutes. From the solution, after standing at room temperature for 24 h, small violet crystals separated were filtered and air-dried, m.p. was 115–120°C. Satisfactory elemental analysis for $C_{48}H_{54}N_4O_{10}Cu$ was obtained. Anal. Calcd (%): C, 63.18; H, 6.10; N, 6.14; Cu, 6.96; H_2O, 3.9. Found (%): C, 63.20; H, 5.84; N, 6.06; Cu, 7 (from atomic absorption determination); H_2O (from thermogravimetric measurement), 5.

2.1.4. Preparation of $[Cu(Carv)(NO_3)\cdot 2H_2O]$ by 2:1 ligand:metal molar ratio. To a solution of carvedilol, CarvH (200 mg, 4.92×10^{-4} mol) in MeOH (7 mL), a solution (3 mL) of $Cu(NO_3)_2 \cdot 3H_2O$ (60 mg; 2.46×10^{-4} mol) in the same solvent was added. The resulting solution was stirred at room temperature for 10 min. From the resulting solution, after standing at room temperature, a blue microcrystalline powder was separated. The powder was filtered and air-dried, m.p. was 130–135°C. Satisfactory elemental analysis for $C_{24}H_{29}N_3O_9Cu$ was obtained. Anal. Calcd (%): C, 50.69; H, 5.20; N, 7.18; Cu, 11.2; H_2O, 6.33. Found (%): C, 50.70; H, 5.10; N, 7.20; Cu, 12 (from atomic absorption determination); H_2O (from thermogravimetric measurement), 6.

2.1.5. Preparation of $[Zn(Carv)Cl]\cdot H_2O$ by 1:1 ligand:metal molar ratio. Carvedilol (60 mg, 1.47×10^{-4} mol) was dissolved in MeOH (15 mL) at 40°C. $ZnCl_2$ (20 mg, 1.47×10^{-4} mol) was added as a solid to the clear solution, under stirring. A white precipitate of the complex was formed instantaneously. Stirring was continued for 30 min, then the precipitate was filtered under vacuum to collect the white solid, which was dried under vacuum for 48 h, m.p. with decomposition was 120–125°C. Satisfactory elemental analysis for $C_{24}H_{27}N_2O_5ZnCl$ was obtained. Anal. Calcd (%): C, 54.97; H, 5.19; N, 5.34; H_2O, 3.43. Found (%): C, 54.08; H, 5.28; N, 5.19; H_2O, 3 (from thermogravimetric measurement).

Reflectance electronic spectra were recorded on a Beckman Acta MIV spectrophotometer. Magnetic susceptibilities were measured at room temperature using a Bruker B-MB4 electrobalance with $Hg[Co(SCN)_4]$ as calibrant and corrected for the diamagnetism of ligand by using the appropriate Pascal's constants.

EPR spectra of polycrystalline samples in DMSO or DMF at 298 and 120 K were obtained in the X-band using a Bruker ER 220D-SRC and a Varian E-9 spectrometer. The magnetic parameters were derived by standardization with diphenylpicrylhydrazyl (dpph). Spin Hamiltonian parameters were obtained by simulating experimental spectra by a revised version of the MONOCLIN program. Estimated errors in the reported $g_\parallel$ and $A_\parallel$ values are ± 0.01 and about $\pm 2 \times 10^{-4}$ cm^{-1}, respectively.

Nuclear magnetic resonance spectra were recorded in a solution of DMSO-d_6, CD_3OD-d_4, or $(CD_3)_2CO$-d_6 on a Varian VX-300 MHz spectrometer at room temperature. Molar ratios from 350 to 1 metal-to-ligand were used. The concentration of the ligand solution was from 1.75×10^{-4} to 1.31×10^{-2} M. The final ligand-to-metal molar ratio of 1:1 was obtained by gradually adding 0.5 µL of metal solution in the appropriate solvent.

3. Results and discussion

Thermoanalyses showed that the compounds lose water or other solvent molecules in the range of 30–100°C, suggesting that solvents are not coordinated to the metal or strongly involved in the coordination complexes, except for $[Cu(Carv)(NO_3)\cdot 2H_2O]$, for which the loss of water molecules is in the range 100–150°C, suggesting such an involvement of solvent molecules in the complex formation (Supplementary material).

Measurements of magnetic susceptibilities for copper complexes at room temperature gave values of μ_{eff} in the 0.7–0.8 MB range, lower than the spin-only value of 1.75–2.22 MB usually found for mononuclear Cu(II) compounds, suggesting an antiferromagnetic coupling between two copper(II) ions in a dimeric structure.

EPR spectra afford complementary information to that of the susceptibility results in that they confirm the binuclear structure of copper compounds, though the typical $S=1$ triplet state transition was not detected. The DMF frozen solution obtained at 120 K of $[Cu(Carv)_2]\cdot 2H_2O$ (**1**) is reported in the "Supplementary material", while in figure 1 the spectrum of $[Cu(Carv)(NO_3)\cdot 2H_2O]$ (**2**) is reported. The spectra of **1** and **2** can be described by an axial spin Hamiltonian. The obtained values $g_{zz} > g_{xx}$, $g_{yy} > 2.040$ suggest σ covalent bonds and a $d_{x^2-y^2}$ ground state, characteristic of an octahedral stereochemistry [16, 18]. The values of $g_{\|}$ and $A_{\|}$ calculated for **1** are in agreement with chromophores in which copper is coordinated to two nitrogens. A superhyperfine structure is visible on the M_I 3/2 component, attributable to the interaction between the unpaired electron of copper and nitrogens of the ligand. Since the superhyperfine patterns are not well resolved in the first derivative experimental spectrum, the second derivative spectrum was computed and simulated and five lines of superhyperfine coupling from two nitrogen atoms can be seen clearly. The simulation which gives the best fit allows the evaluation of the magnetic parameters: $g_{\|}=2.21$, $A_{\|}=180$ G, $A_N=12.5$ G.

From the low temperature EPR spectrum in DMF of **2**, the presence of a dimeric species, though no signals at $g=4$ were obtained, is indicated by the great number of lines in the parallel portion of the spectrum. In this case, the lines from the coupling

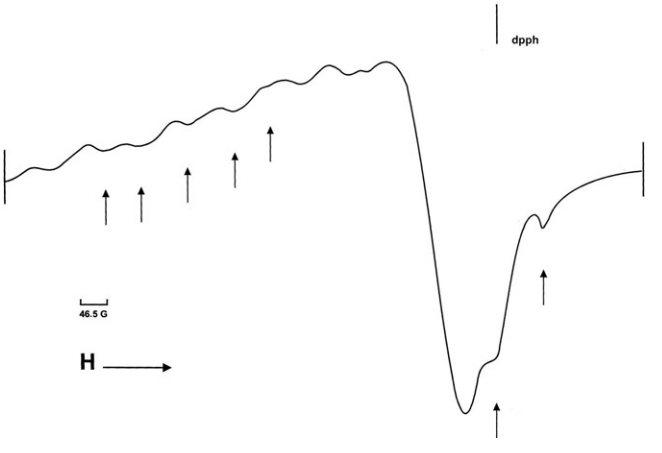

Figure 1. EPR in DMF frozen solution at 120 K of $[Cu(Carv)(NO_3)\cdot 2H_2O]$ (**2**).

with one copper nucleus ($I = 3/2$) are obscured by overlapping of lines due to the dimeric and monomeric species.

These results are in agreement with our previously resolved crystal structure of [Cu(Carv)Cl], which consists of two Cu(II) ions forming a dimer, bridged by two carvedilol ligands. The carvedilol ligand is σ-coordinated to the metal via the N belonging to the amino group and the deprotonated O of propanol of the aliphatic moiety [15].

Such system with electronically interacting pairs of metal ions, which persists for different ligand-to-metal molar ratios as well as for different counter ions, may be important in the biological behavior of the ligand.

The diffuse reflectance electronic spectrum (Supplementary material) of **2** exhibited a very broad band, probably consisting of two overlapped bands, with maximum at 680 nm. The spectrum is in agreement with a 1 : 1 Cu-carvedilol complex and a CuNO chromophore in a distorted tetragonal environment. On passing from Cu : Carv 1 : 1 (compound **2**) to Cu : Carv 1 : 2 (compound **1**), a blue shift in the reflectance electronic spectrum confirms the involvement of two nitrogen donors in coordination to Cu(II). Λ_{max} at 490 and 590 nm as a shoulder is considered diagnostic of a bridging system in a dimeric structure, in agreement with a CuN_2O_2 coordination in a distorted tetragonal environment (Supplementary material) [16, 17].

Thus, all the spectroscopic results are consistent with a tetragonally distorted complex with an in-plane CuNO for $[Cu(Carv)(NO_3) \cdot 2H_2O]$ and CuN_2O_2 chromophore for $[Cu(Carv)_2] \cdot 3H_2O$. EPR spectra of iron compounds gave no information about the coordination environment in which very broad bands have been obtained, indicating a strong dipolar interaction between close metal ions in a high-spin configuration. 1-D NMR of CarvH in CD_3OD for aliphatic and aromatic spectral region are reported in the "Supplementary material". To corroborate the assignments and to resolve the connectivities, 2-D COSY NMR experiments have been carried out in CD_3OD solvent. NMR study in solution, starting from sub-stoichiometric to stoichiometric amount of metals, gives information about iron and zinc coordination to carvedilol. A series of experiments starting from 350 : 1 to 1 : 1 ligand : metal molar ratios have been carried out.

Because of the proton relaxation effects, complexation with a paramagnetic metal ion, such as Fe(III), results in selective broadening of the signals from protons sufficiently close to the binding sites [19]. Due to the rapid exchange between the free and the complexed ligand, a small amount (10^{-4}–10^{-3} M) of paramagnetic ion is able to broaden signals from protons adjacent to the binding site. Significant selective line broadening readily appreciable at a molar ratio as small as 350 : 1 Carv : Fe(III) is induced by the paramagnetic metal through dipole–dipole interactions. There was a general increase in the linewidth of the aliphatic resonances with increasing Fe(III) concentration.

From the NMR spectra it is possible to distinguish two sets of signals, the aromatic resonances were not significantly broadened, while the aliphatic resonances were significantly broadened (Supplementary material). The results obtained from NMR are consistent with the involvement of deprotonated oxygen from C_b carbon and nitrogen donor from aliphatic moiety of the molecule in coordination to Fe(III) as well as to Zn(II). For Cu-bound species relevant shifts of proton signals are not observed, suggesting intermediate exchange phenomena in solution between Cu-bound and free ligand, but for Fe- and Zn-bound species strong shifts of the protons involved in

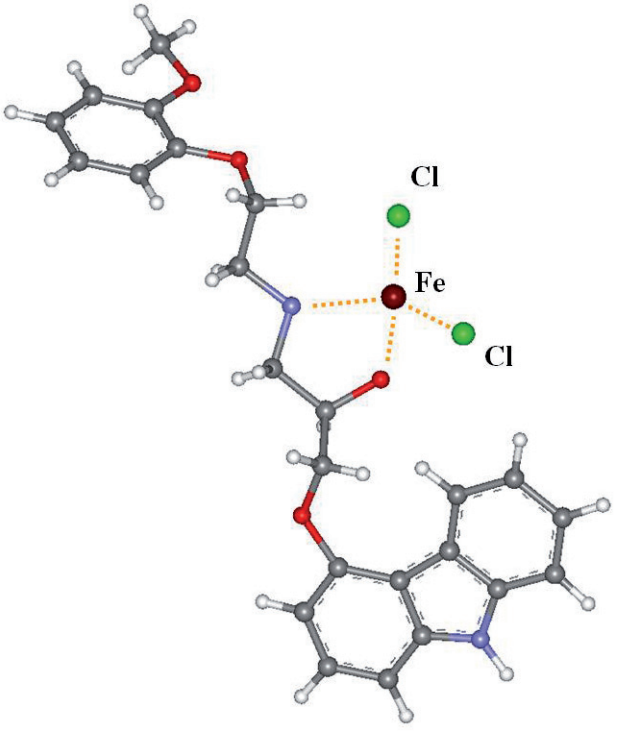

Figure 2. Molecular model of [Fe(Carv)Cl_2].

coordination have been observed, suggesting a strong binding of iron and zinc to the ligand.

A strong downfield shift involves almost all aliphatic protons, indicating binding of metal-to-donors of the aliphatic moiety. For 1 : 1 CarvH : Fe(III) molar ratio, the most affected protons are Hc″ with a $\Delta\delta = +0.33$ ppm (from 3.15 to 3.48 ppm), Hb with a $\Delta\delta = +0.17$ ppm (from 4.31 to 4.48 ppm), and Hc′ with a $\Delta\delta = +0.10$ ppm (from 3.20 to 3.10 ppm). The Ha, He, and Hb signals shift downfield upon coordination, where Ha is least affected. The aromatic signals are not affected by the addition of iron, indicating that the aromatic moiety is far from the coordination center, except for H5 which shows a small upfield shift, suggesting that it is closer than other aromatic protons to the coordination center. The same behavior has been found for copper-bound species for which the structure has been previously resolved [15].

In DMSO, a general increase in the linewidth of the aliphatic resonances with increasing Fe(III) concentration is evidenced. Resonances of hydroxyl OH and amino NH were broadened significantly more than all the other signals and, for 1 : 1 Fe(III) : ligand molar ratio, they disappeared. This behavior confirms that Fe(III) is coordinated through the in-plane O and N donors.

The spectroscopic results allow us to build a model for the iron–carvedilol compound, as shown in figure 2. Because signal disappearance could be from the paramagnetic properties of metals such as iron, NMR studies were also conducted with diamagnetic zinc. Nearly identical NMR results were seen with the diamagnetic ion,

Table 1. NMR of carvedilol and its Zn(II) complex in $(CD_3)_2CO$ at 25°C.

H	CarvH δ (ppm)	J (Hz)	Zn–CarvH complex δ (ppm)	J (Hz)	$\Delta\delta$ (ppm)
NH ar	10.350 (brs)		10.361 (s)		0.011
H_5	8.332 (d)	7.8 ($J_{H5–H6}$)	8.352 (d)	7.5 ($J_{H5–H6}$)	0.02
H_8	7.467 (dt)	8.1 ($J_{H8–H7}$) 0.9 ($J_{H8–H6} = J_{H8–NH}$)	7.477 (d)	8.1 ($J_{H8–H7}$)	0.01
H_7	7.327 (ddd)	8.1 ($J_{H7–H8}$) 7.2 ($J_{H7–H6}$) 1.2 ($J_{H7–H5}$)	7.321 (dd)	8.1 ($J_{H7–H8}$) 7.8 ($J_{H7–H6}$)	−0.006
H_2	7.297 (dd→t)	7.5 ($J_{H2–H1}$) 8.1 ($J_{H2–H3}$)	7.321 (dd)	8.1 ($J_{H2–H1}$) 7.8 ($J_{H2–H3}$)	0.024
H_6	7.135 (ddd)	7.8 ($J_{H6–H5}$) 7.2 ($J_{H6–H7}$) 0.9 ($J_{H6–H8}$)	7.140 (m)		0.005
H_1	7.285 (dd)	7.5 ($J_{H1–H2}$) 0.9 ($J_{H1–H3}$)	7.144 (d)	8.1 ($J_{H1–H2}$)	−0.141
4Hg	6.984–6.821 (sm)		7.126 (dd) (1H)	6.3 6.9	n.d.
			6.980 (d) (2H)	3.9	n.d.
			6.876 (dd) (1H)	3.6 3.9	n.d.
H_3	6.727 (dd)	8.1 ($J_{H3–H2}$) 0.9 ($J_{H3–H1}$)	6.746 (d)	7.8	0.019
H_b	4.312 (m)	7.2 ($J_{Hb–Hc'}$) 4.2 ($J_{Hb–Hc''}$) 1.5 ($J_{Hb–Ha}$)	4.867 (brm)		0.567
OH	4.277 (brs)				1.823
$2H_a$	4.260 (2d)	1.5 ($J_{Ha–Hb}$) 4.8 ($J_{Ha'–Ha''}$)	4.459 (brd)	16.5	0.199
$2H_e$	4.109 (t)	5.4 ($J_{He–Hd}$)	4.378 (br)		0.269
OCH_3	3.774 (s)		3.811 (s)		0.037
$2H_d$	3.068 (m)	1.2 ($J_{Hd–Hc}$) 4.5 ($J_{Hd–NH}$) 5.4 ($J_{Hd–He}$)	3.568 (br)		0.500
$2H_c$	3.099–2.954 (m)	7.2 ($J_{Hc'–Hb}$) 12.2 ($J_{Hc'–Hc''}$) 4.2 ($J_{Hc''–Hb}$)	3.450 (br)		0.423
NH al	2.786 (br)				

suggesting that the signal loss is not from paramagnetic line broadening, but from metal-induced N coordination and hydroxyl deprotonation and coordination.

The assignments for the protons of free ligand and Zn complex in $(CD_3)_2CO$ solvent are reported in table 1. The most affected protons are those of the aliphatic moiety with strong downfield shifts. There was no significant broadening or shifts of aromatic protons, except those from the phenoxy. In fact, while all aromatic phenoxy proton signals are overlapped in the sharp 6.98–6.82 range in the free ligand, they are broadened, separated, and shifted downfield upon zinc coordination.

These results establish that signal broadening is the direct consequence of strong metal binding with N and O donors from the aliphatic moiety and that the phenoxy of carvedilol is positioned towards the metal site upon zinc coordination.

4. Conclusion

All the data point to the chelating coordination of carvedilol towards iron and zinc ions. If our results about sequestering Fe(III) and Zn(II) and previously described Cu(II) ability of carvedilol could be demonstrated in a cellular model, it is possible that this drug could also be used in the therapy of diseases characterized by Fe(III), Zn(II), and Cu(II) accumulation.

Metal ions participate to generate oxygen free radicals through Fenton and Weiber-Weiss reactions [20]. Thus, the ability of carvedilol to sequester metal ions could induce its antioxidative effect. In this context, our results have been obtained in an acellular system and we do not know whether this happens in cells and in organisms. Considering that the extent of reperfusion injury is increased by high tissue iron levels both in experimental models [21] and clinical settings [22], interventions with iron chelators may reduce the reperfusion injury in experimental models [23] and in human myocardium [24]. Considering that serum concentration of carvedilol after an oral dose of 50 mg is about 3 μM [4], and that only the trace of iron is present, it is possible that metal chelation could participate to the antioxidative effect of carvedilol *in vivo*.

In conclusion, our results suggest that carvedilol, 1-[carbazolyl-(4)-oxyl]-3-[2-methoxyphenoxyethyl)-amino]-2-propanol, which is an antihypertensive agent with β-blocker function and antioxidant activity can be considered a drug with chelating properties and, therefore, may be used in the chelation therapy. In a general context of potential activity in biomedical chemistry, it has been reported [25, 26] that the coordination of some drugs by metal ions improved the pharmaceutical activity of the drugs themselves and reduced their undesired toxicity effects in human and veterinary medicine. In our case, the coordination of carvedilol to Fe(III), Zn(II), and Cu(II) can be taken into account in the context of the ability of some drugs to sequester excess of metal ions and thus, in this way, to exert their antioxidant activity.

Acknowledgments

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