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Synthesis, Spectroscopic and Pharmacological Studies of Bivalent Copper, Zinc and Mercury Complexes of Thiourea

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ABSTRACT

A series of metal complexes of Cu(II), Zn(II) and Hg(II) having the general composition $[M(L)_2X_2]$ [where L = thiourea; M = Cu(II), Zn(II) or Hg(II) and X = Cl^-, NO_3^- or CH_3COO^-] have been synthesized. All the metal complexes were characterized by elemental chemical analysis, molar conductance, magnetic susceptibility measurements and IR spectroscopy. Cu(II) complexes were additionally characterized by electronic and EPR spectral studies. The IR spectral data suggests the involvement of sulphur atom in coordination to the central metal ion. On the basis of spectral studies square planar geometry has been assigned to Cu(II) complexes except $[Cu(L)_2(CH_3COO^-)_2]$ which exhibits six coordinated tetragonal geometry. However, all Zn(II) and Hg(II) complexes exhibited tetrahedral geometry. Furthermore, to achieve a better pharmacological profile, thiourea and its metal complexes were screened for *in vitro* antimicrobial activity against the human pathogenic bacteria *Staphylococcus aureus, Staphylococcus epidermides, Escherichia coli* and *Pseudomonas aeruginosa* and against the fungi *Candida albicans* and *Aspergillus niger.* Thiourea and its metal complexes were also screened for *in vivo* anti-inflammatory activity. Thiourea did not exhibit antibacterial activity but showed antifungal activity. However, it is important to note that the Cu(II), Zn(II) and Hg(II) complexes of thiourea exhibited antibacterial and enhanced antifungal activity. Neither thiourea nor its metal complexes exhibited anti-inflammatory activity.

KEYWORDS

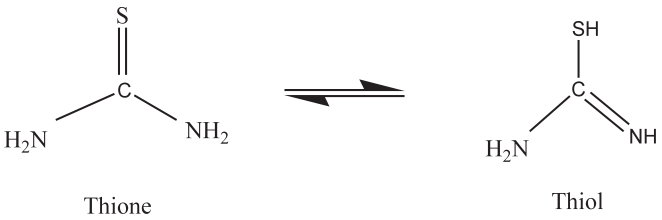
Thiourea complexes, spectroscopy, antibacterial activity, antifungal activity, anti-inflammatory activity.

1. Introduction

Inorganic elements play crucial roles in biological and biomedical processes, and it is evident that many organic compounds used in medicine do not have a purely organic mode of action. Some are activated or biotransformed by metal ions including metalloenzymes. Others have a direct or indirect effect on metal ion metabolism. The field of medicinal inorganic chemistry has been stimulated by the success of cisplatin, the world's best selling anticancer drug.[1] Copper, zinc and mercury metals have a well-established importance in the field of medicine. Copper plays a vital role in the development and performance of the human nervous and cardiovascular system as well as the skin, bone, immune and reproductive system including gene transcription.[2] Copper histidine complex is given as a supplement for Menkes disease treatment.[3] Zinc plays an important role in various biological systems and is a vital component, an essential cofactor, critical for numerous cellular processes and may be a regulatory ion in the metabolism of cells.[4] Zinc citrate is a supplement, ZnO is widely used as a skin ointment and Zn(II) bicyclam complexes possess antiviral activity.[3] Thimerosal is an ethylmercury containing pharmaceutical compound developed in 1927. It continues to remain as an effective preservative in vaccines and other injectable biological products, including Rho(D)–immune globulin preparation.[5]

Ligands containing a thioamide structure have considerable coordination potential. The flexibility based on the tautomerism, thiol (–N=C(–SH)) ⇔ thione (–NH–C(=S)), can afford various coordination modes (Fig. 1). Thiourea is potentially capable of forming coordinate bonds through both sulphur and nitrogen.

Structural studies using spectroscopic techniques (IR and NMR) of thiourea complexes of silver(I), $[Ag((Tu)_2Cl, Ag(Tu)_2SCN$ and $Ag(Tu)_3ClO_4$ where Tu = thiourea] have shown that thiourea can act both as a bridging and terminal ligand.[6] A number of studies on metal coordination compounds of thiourea derivatives with inorganic salts have been reported. They have been found to possess a wide variety of biological activities like antifungal, antibacterial and hypolipidemic activity.[7–9] However, studies dealing with the complexes of thiourea with metal salts and their biological activities are scarce. A variety of compounds containing thiourea can be obtained depending on the method of preparation and also on the anion used. The control of the geometry around a metal ion is important for controlling the properties of a compound. In this paper we report the synthesis, spectroscopic, *in vitro* antimicrobial and *in vivo* anti-inflammatory activity of the Cu(II), Zn(II) and Hg(II) complexes of thiourea.



Thione Thiol

Figure 1 The tautomeric structures of thiourea.

2. Materials and Methods

2.1. Instrumentation

The C, H, and N were analysed on Carlo-Erba 1106 elemental analyser. Molar conductance was measured with the ELICO

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(CM82T) conductivity bridge. The magnetic susceptibilities were measured at room temperature on a Gouy balance using $CuSO_4.5H_2O$ as callibrant. Diamagnetic corrections were made by using Pascal's constants. IR spectra (KBr) were recorded on FTIR spectrum BX-II spectrophotometer. The electronic spectra were recorded in DMSO on Shimadzu UV mini-1240 spectrophotometer. The molecular weights of the complexes were determined cryoscopically in benzene. The EPR spectra of the complexes were recorded at room temperature on a E_4–EPR spectrometer using DPPH as the g-marker.

2.2. Materials

All the chemicals used were of analytical grade and were procured from Sigma Aldrich and Fluka. Metal salts were purchased from E. Merck and used as received.

2.3. General Method of Synthesis of Complexes

An ethanolic solution (20 mL) of thiourea (0.02 mol) and an ethanolic solution (20 mL) of the corresponding metal salt (0.01 mol), heated to a temperature of 50 °C, were mixed together with constant stirring. The mixture was refluxed for 3–4 h at 50–55 °C. On cooling, coloured complexes precipitated. These were filtered off, washed with 50 % ethanol, and dried under vaccum over fused $CaCl_2$.

2.4. *In vitro* Antimicrobial Activity

In vitro antimicrobial screening was performed by the agar disc diffusion method.[10,11] All the test organisms were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. Nutrient agar growth media was prepared according to the instructions of MTCC. 25 mL nutrient agar media was poured into each petriplate of 90 mm diameter. The inoculum was spread on the top of solidified media. Sterile discs of Whatmann no. 1 filter paper, having a diameter of 6 mm, impregnated with the test compounds, were placed at four equidistant places on the inoculated petriplates. The zone of inhibition was calculated in millimeters. The antibacterial activity of the thiourea and its metal complexes were tested against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermides*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogenic bacteria at a concentration of 100 μg disc^{-1}. Nutrient agar media was prepared by using peptone, beef extract, yeast extract, NaCl, agar-agar and distilled water.[12] Bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto the nutrient agar plates.[13] The discs were carefully transferred onto the seeded agar plates. Filter paper disc treated with DMSO served as control and Amikacin (30 μg disc^{-1}) was used as a standard drug. All determinations were made in duplicate for each of the compounds. An average of two independent readings for each compound was recorded. The petriplates were incubated at 37 °C for 24 h before the zone of inhibition was calculated.

The antifungal activity of the thiourea and its metal complexes were tested against two pathogenic fungi, *Candida albicans* and *Aspergillus niger* at a concentration of 200 μg disc^{-1} for each. Nystatin (200 μg disc^{-1}) was used as standard fungicide and DMSO served as a means of control. For *Candida albicans* nutrient agar media was prepared using yeast extract, peptone dextrose, agar-agar and distilled water.[12] Inoculum suspension in normal saline was prepared from fresh, mature (3–5 days old) cultures grown on nutrient agar slants. Using spectrophotometry at 530 nm, turbidity was measured and adjusted to match a 0.5 McFarland density standard resulting in an inoculum containing 1×10^6 to 5×10^6 fungal cells mL^{-1}.[14] This suspension was used to directly inoculate agar plates.

For *Aspergillus niger,* nutrient agar media was prepared using czapek concentrate ($NaNO_3$, KCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ and distilled water), K_2HPO_4, yeast extract, sucrose, agar-agar and distilled water.[12] Seven-day-old colonies were covered with approximately 1 mL of sterile 0.85 % saline and the suspensions were made by gently probing the colonies. The resulting mixture of conidia and hyphal fragments was withdrawn and transferred into sterile tube. After heavy particles were allowed to settle for 3–5 min, the upper homogenous suspensions were collected. The densities of the conidial suspensions were read and adjusted to an optical density (OD) that ranged from 0.09 to 0.11 (80 to 82 % transmittance) at 530 nm.[15] The sterile discs impregnated with the test compounds were placed on the already seeded plates at 30 °C for 48 h. A clearing zone around the disc indicated the inhibition activity of the test compounds on the pathogenic fungi.

2.5. *In vivo* Anti-inflammatory Activity

Anti-inflammatory activity of thiourea and its complexes was determined by carrageenan induced paw oedema model. The animal experimental protocols were approved by the Institutional Animal Ethics Committee (Sanction No: ITS–01/IAEC/2009). Thirty-six Wistar albino rats, 24 females and 12 males, weighing 150–200 g were used. They were kept in polypropylene cages at 25 ± 2 °C under 12 h light and dark cycles. The rats had free access to the rat feed pellets and drinking water. The animals were randomly divided into one control, one reference and four test groups comprising six rats each.

Oedema was induced by injecting 0.1 mL of 1 % w/v carrageenan in 0.9 % NaCl into the subplantar region of the rat's hind paw. The four test groups were treated orally with 10 mg kg^{-1} of test compounds namely thiourea (L), $[Cu(L)_2Cl_2]$, $[Zn(L)_2Cl_2]$ and $[Hg(L)_2Cl_2]$, 30 min before carrageenan injection. The control group received 10 mL kg^{-1} saline and the reference group received 10 mg kg^{-1} indomethacin orally. Measurement of paw size was done 3 h following carrageenan injection. The inhibitory activity was calculated according to the following equation.[16]

$$\text{Percentage inhibition} = \frac{(\text{Ct} - \text{Co}) - (\text{Ct} - \text{Co})\ \text{treated}}{(\text{Ct} - \text{Co})\ \text{control}} \times 100,$$

where Ct is the paw circumference at time t, Co is the paw circumference before carrageenan injection, Ct – Co is oedema, (Ct – Co) control is oedema or paw size after carrageenan injection to control rats at time t.

All the results were expressed as mean ± SEM and the data were statistically analysed by the Student *t*-test, where $P < 0.001$ was considered statistically significant.

3. Results and Discussion

Thiourea behaves as a monodentate ligand coordinating through the sulphur. The analytical data, magnetic susceptibility and spectral analysis agree well with the proposed composition of Cu(II), Zn(II) and Hg(II) complexes of thiourea as shown in Table 1. The molar conductance of the complexes in DMF lies in the range of 12–16 Ω^{-1} cm^2 mol^{-1} indicating their nonelectrolyte behaviour. Thus, the complexes may be formulated as $[M(L)_2X_2]$, (where M = Cu(II), Zn(II) or Hg(II); L = thiourea; X = Cl^-, NO_3^- or CH_3COO^-) (Fig. 2).

3.1. IR Spectra

The assignments of the significant IR spectral bands of thiourea and its metal complexes are presented in Table 2. In

Table 1 Analytical data of the complexes.

Compounds	Mol. Wt found (calcd.) /gm	Yield /%	Colour	Mp /°C	Analysis found (calcd.)			
					C	H	N	M
$[Cu(L)_2Cl_2]$	285 (286)	68	green	>350	8.35 (8.39)	2.76 (2.79)	19.60 (19.56)	22.18 (22.20)
$[Cu(L)_2(NO_3)_2]$	340 (340)	64	green	163	7.06 (7.07)	2.38 (2.35)	24.70 (24.73)	18.73 (18.70)
$[Cu(L)_2(CH_3COO)_2]$	335 (334)	65	green	>350	21.61 (21.59)	4.17 (4.19)	16.75 (16.78)	19.08 (19.05)
$[Zn(L)_2Cl_2]$	290 (288)	60	white	210	8.36 (8.33)	2.79 (2.77)	19.40 (19.44)	22.66 (22.69)
$[Zn(L)_2(NO_3)_2]$	340 (341)	60	white	180	7.00 (7.03)	2.36 (2.34)	24.63 (24.60)	19.13 (19.14)
$[Zn(L)_2(CH_3COO)_2]$	335 (336)	61	white	240	21.46 (21.47)	4.20 (4.17)	16.70 (16.69)	19.46 (19.48)
$[Hg(L)_2Cl_2]$	422 (423)	68	white	210	5.66 (5.67)	1.91 (1.89)	13.24 (13.23)	47.40 (47.39)
$[Hg(L)_2(NO_3)_2]$	475 (477)	62	grey	>350	5.02 (5.03)	1.66 (1.67)	17.60 (17.62)	42.16 (42.14)
$[Hg(L)_2(CH_3COO)_2]$	470 (471)	64	white	>350	15.33 (15.30)	2.96 (2.97)	11.90 (11.89)	42.62 (42.61)

principle, thiourea exhibit thiol-thione tautomerism since it contains a thioamide –NH–C=S functional group. The ν(S–H) band at 2500–2600 cm^{-1} is absent in the IR spectrum of thiourea, but the ν(NH) band, at 3367 cm^{-1}, is present, indicating that, in the solid state, thiourea remains as the thione tautomer.[17] Thiourea exhibits two pairs of asymmetric and symmetric $\nu(NH_2)$ stretching vibrations in the high frequency region of 3400–3000 cm^{-1}. The bands due to $\nu_{as}(NH_2)$ at 3392 and 3273 cm^{-1} and the bands due to $\nu_s(NH_2)$ at 3175 and 3092 cm^{-1} are not shifted to lower frequencies upon formation of the metal thiourea complex, indicating that nitrogen to metal bonds are not present and that the bonding must be between sulphur and metal atoms.[18] The characteristic absorption frequencies of thiourea at 1474 and 1083 cm^{-1} are due to asymmetric and symmetric ν(N–C–N) stretching vibrational modes. These peaks are shifted towards higher frequencies in metal complexes. The increase in the frequency can be attributed to the greater double bond character of the carbon to nitrogen bond on complex formation.[19] The characteristic bands of thiourea at 1415 and 730 cm^{-1} are due to asymmetric and symmetric ν(C=S) stretching vibrations. Upon complex formation, the band of thiourea at 1415 cm^{-1} shows an asymmetric splitting, while the band at

Table 2 Important IR spectral bands (cm^{-1}) of thiourea and its complexes.

Compounds	ν_{as}(N–C–N)	ν_a(N–C–N)	ν_{as}(C=S)	ν_s(C=S)	S–M–S
CH_4N_2S (L)	1474	1083	1415	730	–
$[Cu(L)_2Cl_2]$	1509	1106	1414	696	474
$[Cu(L)_2(NO_3)_2]$	1522	1093	1390 1415	636	478
$[Cu(L)_2(CH_3COO)_2]$	1504	1107	1320 1434	723	477
$[Zn(L)_2Cl_2]$	1483	1093	1408 1439	709	476
$[Zn(L)_2(NO_3)_2]$	1496	1105	1389 1446	715	477
$[Zn(L)_2(CH_3COO)_2]$	1504	1107	1407 1434	725	477
$[Hg(L)_2Cl_2]$	1503	1110	1408 1438	714	479
$[Hg(L)_2(NO_3)_2]$	1522	1109	1406 1420	618	473
$[Hg(L)_2(CH_3COO)_2]$	1522	1112	1395 1381 1325	697	473

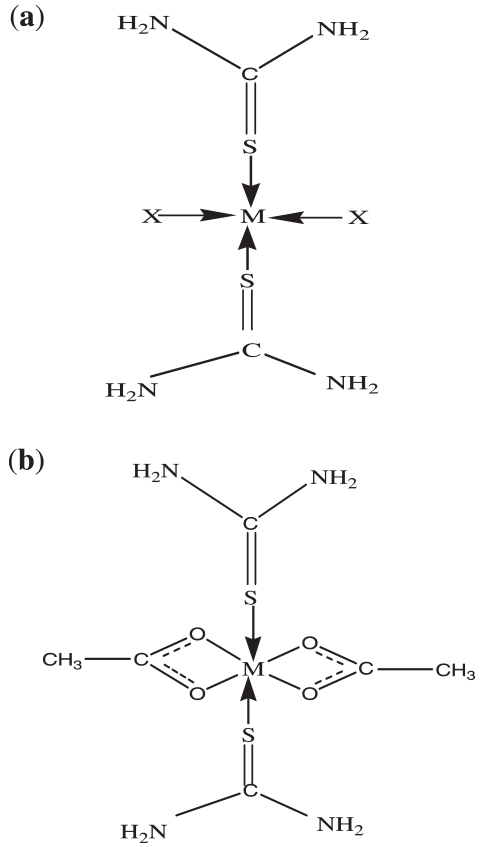

Figure 2 Suggested structure of the complexes. (**a**) $[M(CH_4N_2S)_2X_2]$, where M = Cu(II), Zn(II) or Hg(II) and X = Cl^- or NO_3^-; $[Zn(CH_4N_2S)_2(CH_3COO)_2]$ or $[Hg(CH_4N_2S)_2(CH_3COO)_2]$. (**b**) $[Cu(CH_4N_2S)_2(CH_3COO)_2]$.

703 cm^{-1} shifts towards lower frequency. The coordination of thiourea ligand to copper, zinc and mercury atoms is realized through the sulphur atom in the complex by observed lowering of ν(C=S) frequencies attributed to the reduced double bond character of the ν(C=S) band.[20] Metal sulphur bonds present in the compound is also evident from the peaks at 479–473 cm^{-1} in the complexes due to antisymmetric S–M–S stretching as well as C–N deformation.[21] In each complex, two thiourea ligands coordinate to the central metal ion through two S atoms. Thus, it is concluded that the ligand acts as a unidentate chelating agent

In the IR spectra of chloro complexes, bands corresponding to ν(M–Cl) are observed at 345–320 cm^{-1} indicating the pres ence of an M–Cl bond.[22] The presence of bands at 1420–1415, 1320–1280, and 1051–1018 cm^{-1}, in the IR spectra of the nitrato complexes suggests that both nitrate groups are coordinated to the central metal ion in a unidentate manner.[23] IR spectra of the Cu(II) acetate complex shows two absorption bands at 1646 and 1504 cm^{-1} which are assigned to $\nu_{as}(CO_2^-)$ and $\nu_s(CO_2^-)$ of CH_3COO^-ion. The stretching frequency difference $[\nu(\nu_{as} - \nu_s) = 142\ cm^{-1}]$ is smaller than that of CH_3COONa (*ca.* 195 cm^{-1}), indicating that the coordination of CH_3COO^-ion is bidentate.[24] IR spectra of Zn(II) and Hg(II) acetate complexes show medium intensity bands at 1620–1618 and 1325–1322 cm^{-1}. These bands are mainly due to monodentate CH_3COO^-.[23]

3.2. Magnetic Susceptibility

As shown in Table 3, magnetic moment observed for the Cu(II) complexes lies within the range of 1.90–1.94 BM, corresponding to one unpaired electron.

3.3. Electronic Spectra

The electronic spectra (Table 3) of $Cu(L)_2Cl_2$ and $Cu(L)_2(NO_3)_2$ complexes show absorption bands at 19010 and 19380 cm^{-1}, respectively, due to $^2B_{1g} \rightarrow {}^2E_g$ transition in a square planar geometry[25]. The bands at 24038 and 24271 cm^{-1} respectively may be attributed to M → L charge transfer.[26] The electronic spectra of $Cu(L)_2(CH_3COO)_2$ shows an absorption band at 10438 cm^{-1}. This band correspond to the transition $^2B_{1g} \rightarrow {}^2A_{1g}$ ($d_{x^2-y^2} \rightarrow d_{z^2}$) in a tetragonal geometry, while the band at 29586 cm^{-1} may be attributed to M → L charge transfer.[27]

3.4. EPR Spectra

Room temperature EPR spectra of Cu(II) complexes (Figs 3–5) were recorded as polycrystalline samples at room temperature. The $g_\parallel$ and $g_\perp$ have been calculated and observed in the range 2.2498–2.2576 and 2.0641–2.0773, respectively (Table 3). These data support the idea that $d_{x^2-y^2}$ may be the ground state ($g_\parallel > g_\perp > 2.0023$) (16). $G = (g_\parallel - 2)/(g_\perp - 2$, which measures the exchange interaction between the metal centres in a polycrystalline solid, has been calculated. According to Hathaway[28], if G > 4, the exchange interaction is negligible, but G < 4 indicates considerable exchange interaction in solid complexes. All the Cu(II) complexes reported in this paper give the '*G*'

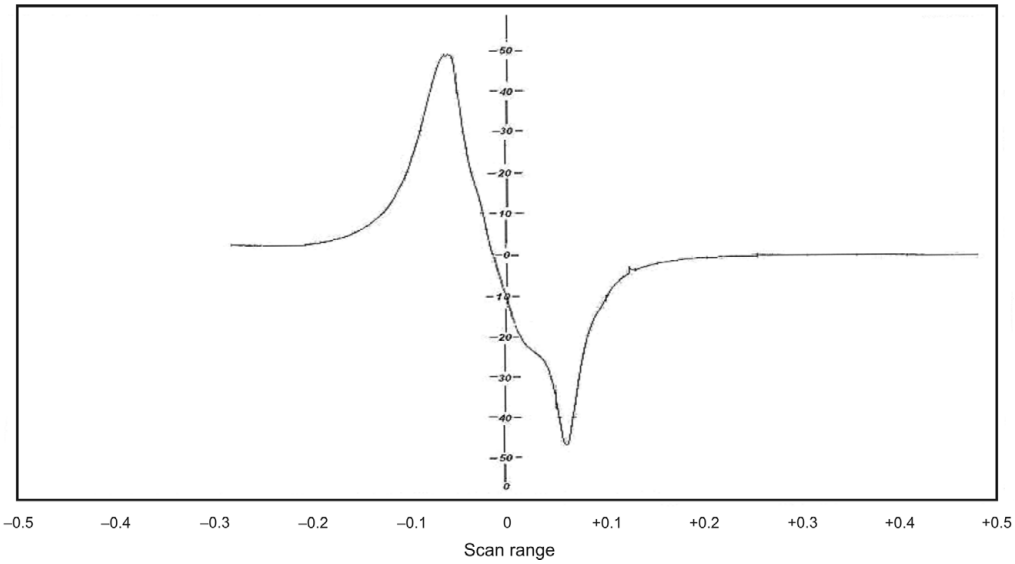

Figure 3 EPR spectra of $[Cu(L)_2Cl_2]$.

Table 3 Magnetic moment, electronic and EPR spectral data of the copper complexes.

Complexes	μ_{eff} BM	λ_{max} /cm^{-1}	g∥	g⊥	G
$[Cu(L)_2Cl_2]$	1.85	19010, 24038	2.0773	2.257	3.3324
$[Cu(L)_2(NO_3)_2]$	1.83	19380, 24271	2.0641	2.249	3.8970
$[Cu(L)_2(CH_3COO)_2]$	1.89	10438, 29586	2.0706	2.2498	3.5382

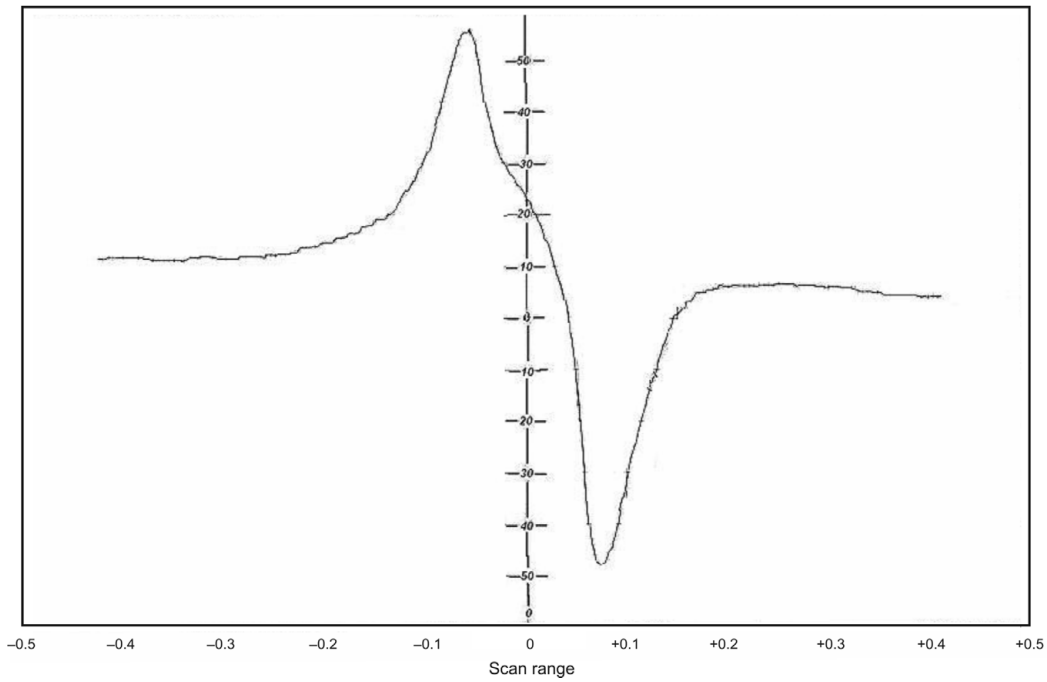

Figure 4 EPR spectra of $[Cu(L)_2(NO_3)_2]$.

values < 4, indicating the exchange interaction in the solid complexes.

3.5. *In vitro* Antimicrobial Activity

The antimicrobial screening data given in Table 4 show that thiourea did not inhibit growth of bacteria but exhibited antifungal activities. It is important to know that it's metal chelates exhibited antibacterial activities against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermides*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogenic bacteria and showed increased antifungal activities against *Candida albicans* and *Aspergillus niger*. $[Cu(L)_2Cl_2]$, $[Zn(L)_2Cl_2]$ and $[Hg(L)_2Cl_2]$ are found to be the most active amongst the series of Cu(II), Zn(II) and Hg(II) complexes synthesized.

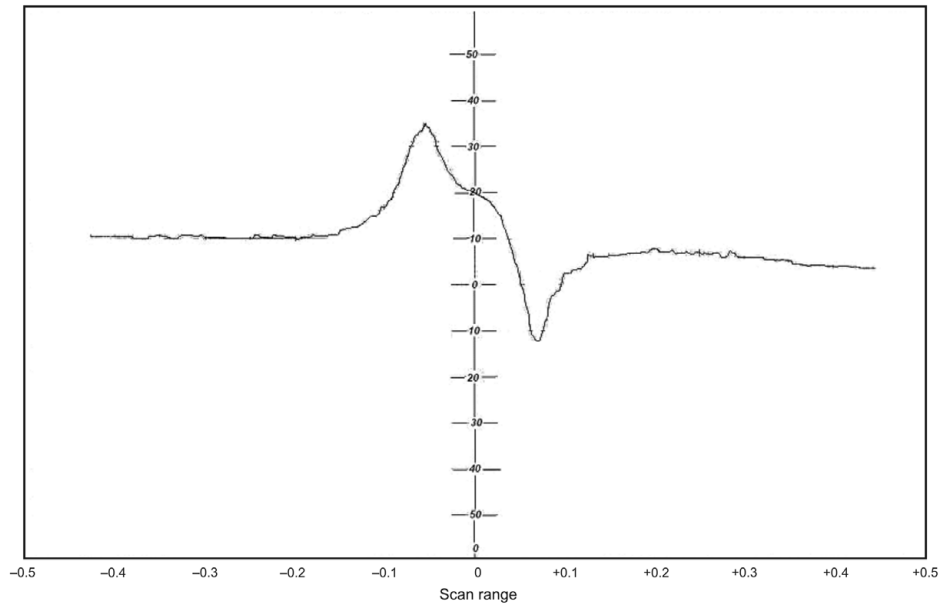

Figure 5 EPR spectra of $[Cu(L)_2(CH_3COO)_2]$.

Table 4 Antibacterial activity of Thiourea and its complexes.

Compound	Diameter of zone of inhibition /mm			
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Thiourea	–	–	–	–
$CuCl_2$	13	11	14	13
$Cu(NO_3)_2$	10	10	11	09
$Cu(CH_3COO)_2$	10	09	10	08
$Zn\ Cl_2$	11	10	13	12
$Zn(NO_3)_2$	09	08	12	10
$Zn(CH_3COO)_2$	10	09	10	08
$Hg\ Cl_2$	11	09	09	10
$Hg(NO_3)_2$	10	08	11	09
$Hg(CH_3COO)_2$	10	09	08	08
Amikacin	30	26	22	21

Table 5 Antifungal activity of Thiourea and its complexes.

Compound	Diameter of zone of inhibition /mm	
	Candida albicans	*Aspergillus niger*
Thiourea	11	08
$CuCl_2$	15	12
$Cu(NO_3)_2$	14	11
$Cu(CH_3COO)_2$	13	11
$Zn\ Cl_2$	14	11
$Zn(NO_3)_2$	12	10
$Zn(CH_3COO)_2$	12	09
$Hg\ Cl_2$	12	09
$Hg(NO_3)_2$	12	08
$Hg(CH_3COO)_2$	11	09
Nystatin	26	18

Formation of complexes with transition metal ions has been proposed as a step in the antimicrobial activity of thiourea. The increased activity of metal chelates can be explained on the basis of chelation theory.[29] It is known that chelation tends to make the ligand act as a more powerful and potent bactericidal agent. It is observed that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligand, and there may be π electron delocalization over the whole chelate. This increases the lipophilic character of the metal chelate and favors its permeation through the lipoid layer of the bacterial membranes. There are other factors which also increase the activity, namely solubility, conductivity, and bond length between the metal and the ligand.

Table 6 Anti-inflammatory activity of Thiourea and its complexes.

Group	Initial paw size [a] /cm	Paw oedema [a] /mm	Inhibition /%
Control	2.4 ± 0.05	5.3 ± 0.51	–
CH_4N_2S (L)	2.4 ± 0.08	5.5 ± 0.54	–
$[Cu(L)_2Cl_2]$	2.5 ± 0.05	5.6 ± 0.51	–
$[Zn(L)_2Cl_2]$	2.3 ± 0.15	5.3 ± 0.51	–
$[Hg(L)_2Cl_2]$	2.4 ± 0.10	5.5 ± 0.54	–
Indomethacin	2.4 ± 0.08	1.3 ± 0.51$^+$	75.04

$^+$ $P < 0.001$ compared with control; Student's *t*-test.
[a] Each value is the mean ± SEM of six rats.

3.6. *In vivo* Anti-inflammatory Activity

The anti-inflammatory screening data given in Table 6 show that thiourea and its metal complexes do not exhibit anti-inflammatory activity

4. Conclusion

The proposed study revealed tetrahedral geometry for Zn(II) and Hg(II) complexes whereas square planar geometry for Cu(II) complexes except $[Cu(L)_2(CH_3COO)_2]$ which possesses six coordinated tetragonal geometry. Thiourea acts as monodentate chelating agent coordinating through the sulphur atom. It is important to note that thiourea did not inhibit growth of bacteria but exhibited antifungal activity. However Cu(II), Zn(II) and Hg(II) complexes of thiourea exhibited antibacterial and enhanced antifungal activity. Neither thiourea nor its metal complexes exhibited *in vivo* anti-inflammatory activity. Cu(II) complexes were found to be biologically more active than Zn(II) and Hg(II) complexes. Moreover, the presence of chloride ions in the metal complexes improves their antimicrobial activity due to formation of hypochlorous acid.[30] Free chloride upon oxidation results into chlorine that reacts with water according to the following proposed reaction:

$$Cl_2 + H_2O \rightarrow HCl + HClO.$$

The hypochlorous acid formed, further decomposes forming hydrochloric acid.

$$2HClO \rightarrow 2HCl + O_2.$$

The O_2 released in the above reaction is a strong oxidizing agent and it destroys microbes by oxidizing cellular components. Antimicrobial action of chlorine compounds is also due to the combination of chlorine with proteins and enzymes of membranes.

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