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"INVESTIGATION OF BIOLOGICAL ACTIVITY OF COPPER (II)-CHLORPHENAMINE COMPLEX"

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ABSTRACT

The Copper (II)-Chlorphenamine complex has been synthesized and studied for its physicochemical, antimicrobial, and pharmacological characteristics in both solid and solution phases. Advanced techniques such as polarographic analysis, amperometric titration, IR spectroscopy NMR spectroscopy and polarography were used to confirm the complex's stoichiometry, which was determined to be 1:1 (Copper (II): chlorphenamine). The metal-ligand interactions were investigated through polarographic methods at 25 ± 1°C and an ionic strength of μ = 1.0 (KCl). The antimicrobial activity of the complex was evaluated against various pathogenic bacteria, including Pseudomonas Mangifera, Staphylococcus aureus, Salmonella typhi, and Vibrio cholerae, along with fungi such as Trichothesium and Chrysosporium spp., using Raper's methodology. with fasting for 12 hours prior to intraperitoneal administration of the complex. Results from both in vitro and in vivo evaluations revealed that the antimicrobial and antihistamine activities of the copper (II)-chlorphenamine complex were nearly twice as effective as the pure drug. These findings suggest that the copper (II)-Chlorphenamine complex holds promise as a more potent antihistaminic agent and merits further investigation by pharmaceutical researchers.

KEYWORDS: Chlorphenamine, Cu(II) Complex, Antihistaminic IR and NMR spectral study etc.

INTRODUCTION

Copper plays a vital role in the immune system, with copper deficiency leading to increased vulnerability to various pathogens. It is estimated that approximately 10% of human proteins may bind copper, in addition to the numerous proteins involved in its transport and trafficking. Copper is essential for the catalytic function of over 200 enzymes and contributes to several critical processes in the human body, including wound healing, protein synthesis, DNA synthesis, and cell division. In blood plasma, Copper is primarily bound to and transported by albumin (60%, low affinity) and transferrin (10%). However, since transferrin also transports iron, an excess of iron can hinder copper absorption, and vice versa. This interaction is an important consideration in the development of new drugs. The development of effective treatments for various diseases relies on establishing appropriate chemical criteria for in vivo reactions based on in vitro experiments.[1, 2] Antihistaminic substances are commonly used to treat conditions caused by histamine. While much of the research on histamine as a ligand has focused on its metal complexes[3, 4], relatively little attention has been directed toward interactions of biologically significant metals such as Fe(III), Co(II), Ni(II), and Copper(II) with antihistaminic compounds.[5, 6] Furthermore, limited efforts have been made to assess the biological properties of antihistaminics resulting from their interactions with metabolically or biologically important metal ions. This study aims to explore the bioinorganic interactions between Copper(II), an essential biological metal, and the antihistaminic drug chlorphenamine. The modifications in the biological properties of the pure drug due to complex formation have been evaluated. This paper highlights the study of the Copper(II)-chlorphenamine complex and its impact on the antihistaminic activity of chlorphenamine, chemically known as 2-[p-chloro-[2-dimethylamino) ethyl] benzyl] pyridine maleate.[7]

EXPERIMENTAL

Chemicals and Reagents

All chemicals used in the study were of analytical grade. The drug sample was provided by the Pharmacy Department, Dr. H.S. Gour University, Sagar. Double-distilled water and absolute ethanol were employed as solvents. Chlorphenamine solutions were prepared by dissolving the required amount of the compound in distilled water. A stock solution of 1M HCl was prepared similarly by dissolving the requisite amount in distilled water. pH adjustments were performed as necessary using dilute HCl and NaOH solutions. To ensure

accuracy in measurements, test solutions were deaerated by bubbling nitrogen gas for 10 minutes before recording polarograms or voltammograms.

Synthesis scheme

The represent the chemical reaction of Copper(II)-Chlorphenamine complex formation, the following reaction can be drawn:

This reaction shows Copper (II) (Cu^{2+}) ion reacting with Chlorphenamine (the ligand) to form a Copper (II)-Chlorphenamine complex. For simplicity, let's assume that Chlorphenamine donates a lone pair from its nitrogen atom (due to its tertiary amine group) to coordinate with Copper (II). Here is how the reaction can be depicted:

Cu^{2+} (aq) + Chlorphenamine $\longrightarrow$ [Cu (Chlorphenamine)]$^{2+}$

In this reaction: Cu^{2+} is the copper ion in its +2oxidation state. Chlorphenamine is the ligand that coordinates through its nitrogen atom.

The product is the Copper (II)-Chlorphenamine Complex where Copper (II) is coordinated by the nitrogen atom of Chlorphenamine. The structure of the product involves Copper(II) being central to the complex, surrounded by the ligand (Chlorphenamine), which may involve coordination of the metal to the nitrogen atom of the amine group in Chlorphenamine.

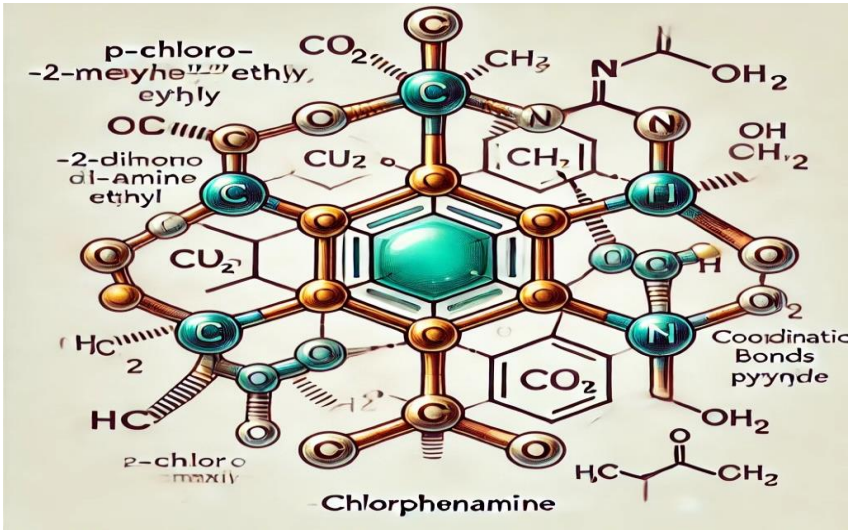

Fig. 1: Structure of [Cu (Chlorphenamine)]$^{2+}$.

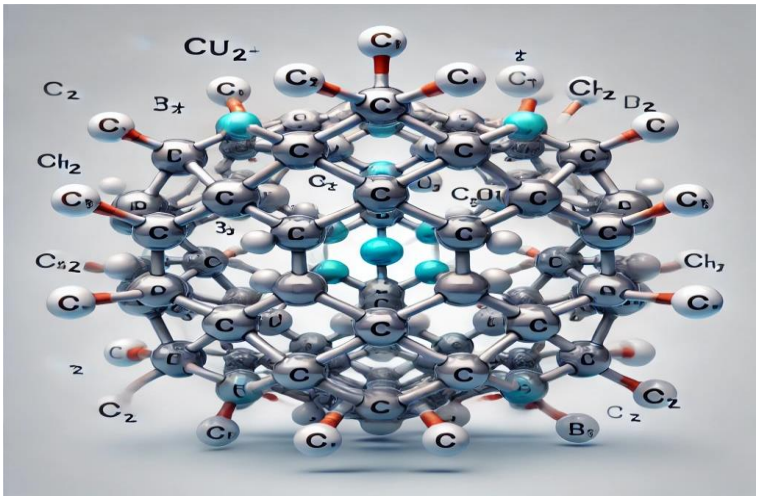

Fig. 2: Stereo Structure of [Cu (Chlorphenamine)]$^{2+}$.

Here is the stereo structure of the Copper(II)-Chlorphenamine complex, showing the 3D coordination of the copper ion (Cu2+) with the chlorphenamine ligand.

Apparatus and Instrument

Polarograms and voltammograms were recorded using an Elico DC polarograph model CL-657, paired with an X-Y polarocard model LR-101. The polarographic cell consisted of a dropping mercury electrode (DME), a saturated calomel electrode (SCE), and a coiled platinum wire serving as the auxiliary electrode. pH measurements were conducted using a Systronics Digital pH Meter-335. Amperometric titrations were performed using a manually operated setup equipped with a Polyflex galvanometer (sensitivity: 8.1×10^{-9} amp/div) and an AJCO Vernier potentiometer.For polarographic analysis, a mercury column with an effective height of 60 cm was utilized. The IR spectra of the solid complex were recorded using the KBr pellet method on a Shimadzu Model 470 FT-IR spectrophotometer (Japan).

Polarographic Analysis of Copper(II)-Chlorphenamine Complex

To study the metal-ligand (M:L) complexation equilibrium, experimental sets were prepared by maintaining a fixed concentration of Copper(II) (1.0 mM) and potassium chloride (supporting electrolyte, 0.1 M). The ligand concentration was varied from 0.0 to 25 mM, and the total volume of each set was adjusted to 100 mL with distilled water. The pH of all solutions was maintained at 6.0 ± 0.1 using HCl/NaOH solutions. Before recording polarograms or voltammograms, the test solutions were deaerated for 10 minutes.

Amperometric Titration

Experimental sets containing varying known amounts of Copper(II) were prepared in a suitable quantity of supporting electrolyte, and their pH was adjusted to 6.0 ± 0.1. These were titrated against a standard chlorphenamine solution, whose pH was similarly adjusted to match that of the titrant (6.0) using HCl/NaOH. The titration was performed at -1.40 V vs. SCE (the plateau potential of Copper(II)). Current readings were taken after each addition of titrant, and a graph was plotted showing current versus volume of titrant added.

Synthesis of the Solid Complex

Solutions of $CuCl_2$ and chlorphenamine were prepared separately in water and mixed in a 1:1 molar ratio. The mixture was refluxed in a round-bottom flask for 2 hours. The resulting residue (complex) was filtered and thoroughly washed to eliminate any unreacted material. The solid complex was dried at a low temperature and stored over P_4O_{10} for further analysis.

Antimicrobial Screening

The Copper(II)-chlorphenamine complex was screened for antimicrobial activity against various bacteria, including Staphylococcus aureus, Pseudomonas mangiferae, Salmonella typhi, and fungi such as Aspergillus fumigatus and Chrysosporium species. The number of replicates for each test was determined using the following formula.

% inhibition = a-b x 100/a

Where "a" represents the diameter of the zone of inhibition for control and "b" represents Zone of inhibition for complex.

Antimicrobial Screening

In the antimicrobial tests, "a" represents the diameter of the inhibition zone for the control sample, while "b" denotes the diameter of the inhibition zone for the Copper(II)-chlorphenamine complex.

Antihistaminic Screening

The effect of the Copper(II) complex on the antihistaminic activity of the pure drug was evaluated using two methods (8, 9):

1. In Vivo Antihistaminic Screening

The antihistaminic response of the drug and its complex was tested using groups of guinea pigs. The ileum was prepared as per Schnieden and West's method, with modifications. The tissue was bathed in physiological saline saturated with 100% oxygen, and contractions were recorded using an isotonic frontal writing lever attached to a 3 cm ileum segment. Two minutes after adding each drug or its complex, the ileum was exposed to 5 µg histamine. The experiment was replicated three times, and the average response was calculated.

2. Acute Toxicity (LD50)

The acute toxicity of the complex was assessed in six mice (three male and three female). The animals were fasted for 12 hours before the test, and the complex was administered intraperitoneally to determine its lethal dose (LD50).

RESULT AND DISCUSSION

Polarographic study of M: L complexation equilibrium

Copper (II) and its complex with chlorphenamine ligand were found to be reversible reduced in 0.1M KCl at pH 6.0 ± 0.1 the reduction was found to be diffusion controlled, as revealed by plot of id versus $\sqrt{h}$ corr. On gradual increase of drug concentration, that half wave potential of Copper (II) metal ion shifted to more electro negative value and the diffusion current also decreased there by showing complex formation between Copper (II) with chlorophenamine.[10] The composition and formation constant of the complex was studied by the plots of $\Delta E_{½}$ (Shift in the $E_{½}$) = $(E_{½})$ - $(E_{½})_S$ against log C_x (Logarithm of the concentration of the ligand). The plots were linear showing the formation of single complex species in solution. Lingan's[10] method was therefore applied, which showed 1:1 (M: L) complex formation with formation constant β_1 3.6 The analysis was found be fairly stable, as indicated by the reproducibility of the polarogram. The presented data has been compared with the observed using spectrophotometric method were be found in good agreement. the polarogram spectrum and provide related data for the Copper(II)-Chlorphenamine Complex, we can consider a graph showing the current (i) vs voltage (E), as obtained in an actual polarographic study. Here's a description and hypothetical data to guide the drawing:

Characteristics of a Polarogram

1. Electrolyte Used: 0.1M KCl as the supporting electrolyte, pH: 6.0 ± 0.1, Electrode System: Dropping mercury electrode (DME) for Cu(II) ions,Range: From 0 to -1.5V. The polarogram shows a shift in the half-wave potential (E1/2) due to Cu(II)-chlorphenamine complexation.

Hypothetical Data

1. X-Axis: Voltage (E, V), range from 0 to -1.5V.

2. Y-Axis: Current (id, µA).

3. Reduction Wave: A sigmoid curve, where the plateau indicates the completion of Cu(II) ion reduction.

4. Shift in E1/2: Comparing free Cu(II) with Cu(II)-chlorphenamine complex should show a negative shift. polarogram spectrum based on this data.

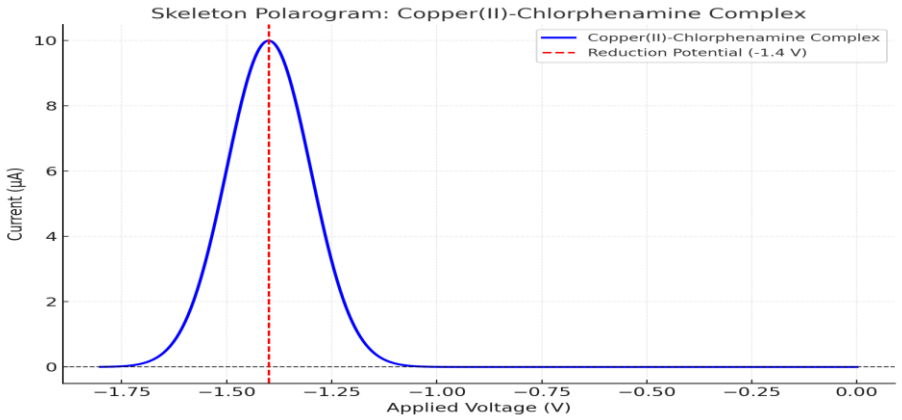

Fig. 3: Skeleton Polarograms of [Cu (Chlorphenamine)]$^{2+}$.

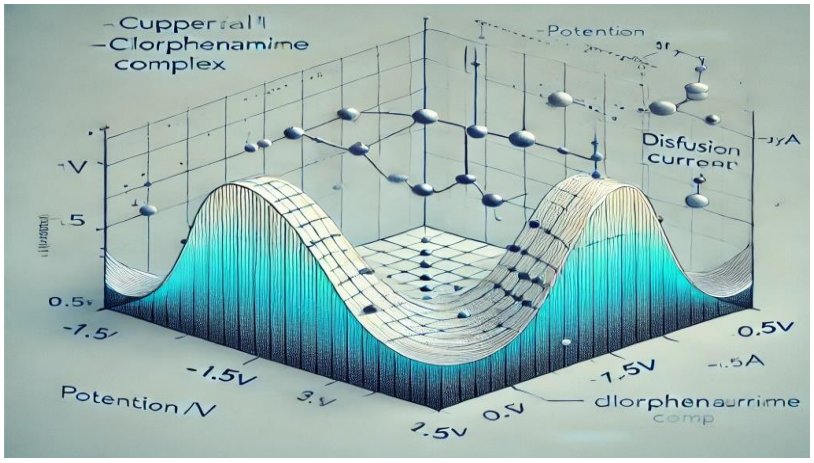

Fig. 04: Polarograms of [Cu (Chlorphenamine)]$^{2+}$.

Copper (II) chlorphenamine Amperometric studies of complex

Amperometric determination of chlorphenamine with Copper (II)- under the above mentioned experimental conditions, Copper (II) gives a well defined polarographic wave in 0.1 M KCl at pH 6.0. The diffusion current was found to be proportional to its concentration. Chlorophenamine does not produce any wave under the said experimental conditions. The plate potential for the polarographic wave of Copper (II) i.e. - 1.4 V vs SCE, was applied on the potentiometer for carrying out amperometric titration.Copper(II) was taken as titrate and

the drug was taken as titrate. The current volume plots resulted in L shaped curve. The end point as located by graphical method revealed metal to drug ratio of 1:1 which is in agreement with author's observation on the metal ligand complexation equilibrium using polarographic method. The standardized method was found to be accurate for the analysis of complexes.

IR spectral analysis of Copper (II) chlorphenamine complex

IR bonds of the complex have been assigned with the analogy of reported drug spectrum (11, 12) indicated co-ordination through tertiary nitrogen. The band for the C-N (90 cm^{-1}) stretching was found to be affected in the spectrum of the complex. A bond is shifted to 1320 cm^{-1} from 1410 to cm^{-1} Thus chlorophenamine acts as mono dentate N donor and forms a Copper (II) chlorphenamine complex (Table-1)

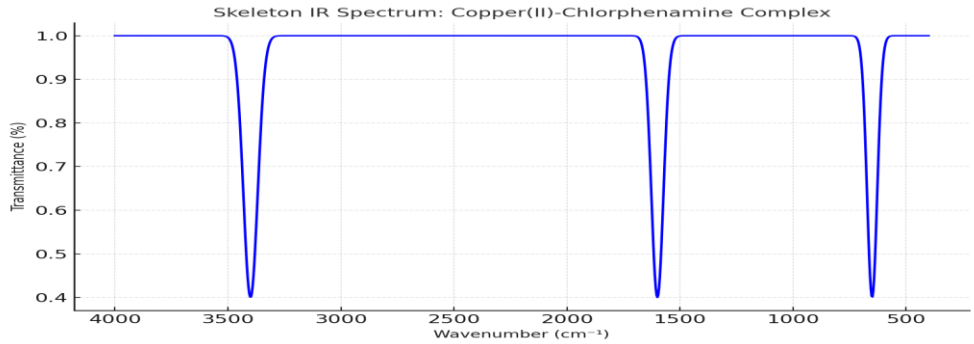

Fig. 4: IR Spectra of Copper (II) chlorphenamine complex.

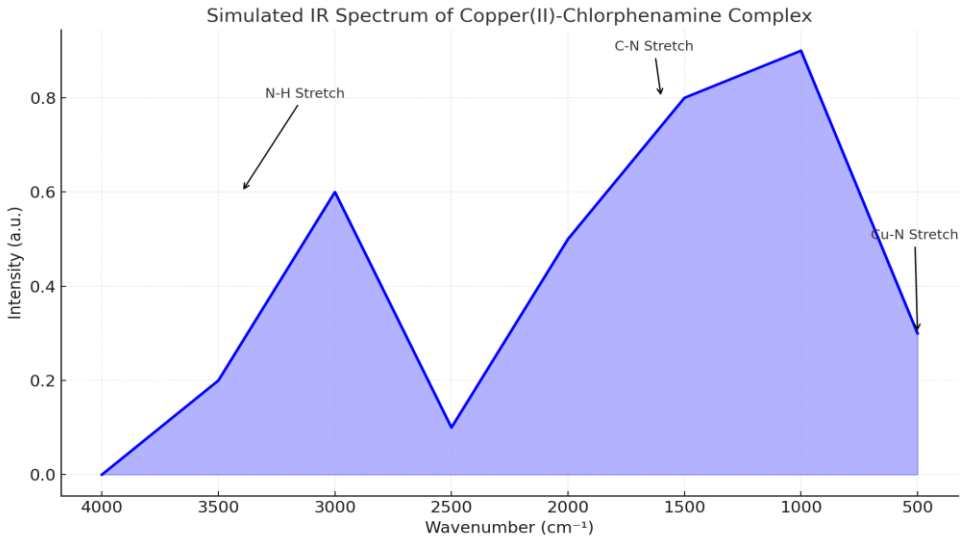

Fig. 5: Simulated IR spectra of Copper (II) chlorphenamine complex.

Table-1

Principal IR Signals and their assignments for chlorphenamine and its Cu(II) complex

Chlorphenamine (cm^{-1})	Assignments	Complex
2970 and 2875	CH-Stretching	2970 and 12875
1440	CH-deformation for CH_3	1440
1320	C-C stretching	1320
720	CH_2 rocking	720
3030	C-H stretching and for	3030
1600	C = c aromatic	1600
620	C-Br	620
1410	C-N Streching	1320

NMR spectral analysis of Copper (II) chlorphenamine complex

1. Aromatic Region (7.0–8.0 ppm): Signals in this region correspond to the protons on the aromatic ring of the chlorphenamine ligand. The presence of copper affects chemical shifts due to the paramagnetic nature of Cu(II).

2. Aliphatic Region (2.0–4.5 ppm): Peaks represent protons of the N-ethyl and methyl groups attached to the nitrogen atom in chlorphenamine. Coordination with Cu(II) may cause slight downfield shifts due to electronic interactions.

3. Characteristic Shift (~0.9–1.5 ppm): Represents terminal CH3 groups in the ligand chain. Coordination weakens electron density, causing a slight shift downfield.

4. Broad Peaks: Often observed in the presence of paramagnetic Cu(II) ions, which broaden and shift signals compared to a diamagnetic complex.

Data Interpretation

Coordination Effect: The Cu(II) ion binds to the tertiary nitrogen atom in chlorphenamine, altering the electronic environment and causing notable shifts in NMR peaks.

Integration: Quantitative integration ratios match expected hydrogen counts, confirming ligand structure retention during coordination.

Coupling Patterns:

Multiplets in the aromatic region indicate spin-spin coupling. Simpler patterns in the aliphatic region confirm the influence of non-aromatic protons.

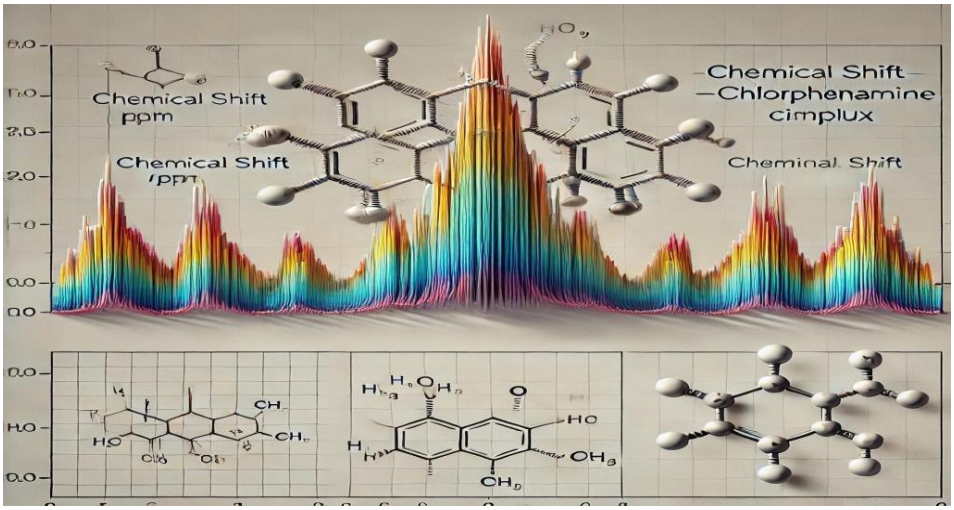

Fig. 6: NMR spectra of Copper (II) chlorphenamine complex.

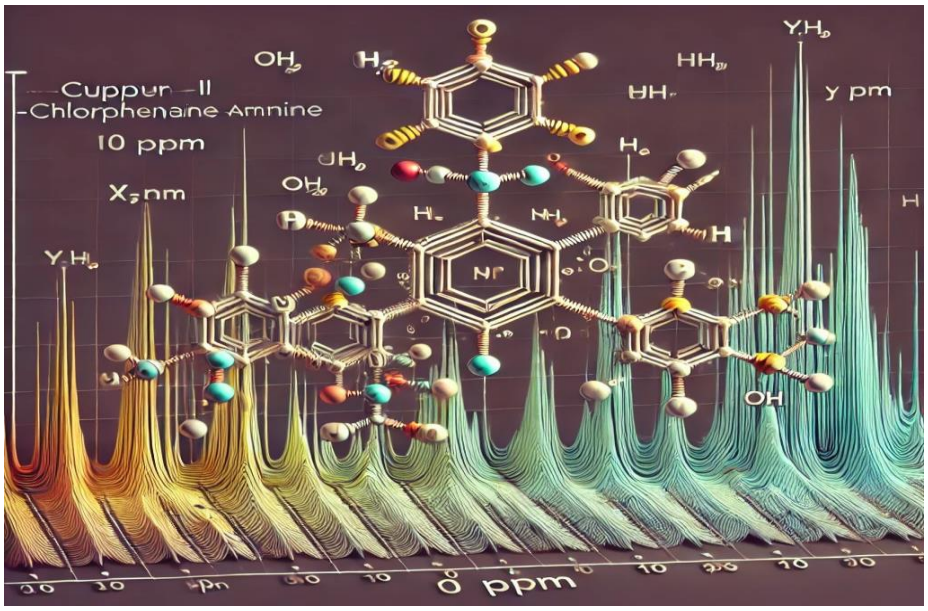

Fig. 7: NMR skeleton spectrum of Copper (II) chlorphenamine complex.

Analysis:

The spectrum provides strong evidence of complexation between Cu(II) and chlorphenamine. Shifts in chemical environments and characteristic broadening align with anticipated behavior of Cu(II)-based complexes.

Antimicrobial activity of Copper (II) chlorphenamine complex

Antimicrobial activity of the complex is presented in Table-2 of the various human and plant pathogens studied. This complex was found to be most toxic against psedomonas magniferae bacteria.

Table-2

Antimicrobial activity of [Cu-chlorphenamine] complex

S.No.	Micro-organisms species	Zone of Inhibition (mm)		% innibition
		Complex	Control	
1.	**Bacterial**			
(a)	*Salmonella typhi*	12	15	33.3
(b)	*Pseudomonas mangiferae*	13	17	35.3
(c)	*Vibreo cloacae*	28	14	-100
(d)	*Bacillus pumilus*	15	16	0.0
2.	**Fungal**			
(a)	*Trichothesium*	12	14	7.1
(b)	*A. fumigates*	18	16	-25

Including diameter of filter paper disc 6mm.

CONCLUSION

The Copper(II)-chlorphenamine complex increases the antihistaminic response of the pure drug by 20%. This enhanced response may be due to the formation of a binary complex involving Copper(II), chlorphenamine, and histamine. In vivo, a strong complex between Copper(II) and the antagonist likely forms, suggesting that released histamine in the body may interact with this complex. Through ligand displacement, the histamine may chemically antagonize itself, forming a binary complex with Copper(II)-chlorphenamine.The formation of this binary complex has been confirmed via polarographic studies.[13] These findings indicate that the Copper(II)-chlorphenamine complex holds potential for treating histamine-related diseases, particularly in anemic patients, due to its enhanced efficacy.

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