Synthesis, Characterization, and Pharmacological Evaluation of Metal-Chelating Compounds for Neuroprotection in Oxidative Stress-Induced Neuronal Damage

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Abstract

Background:

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are associated with oxidative stress and metal ion dysregulation, leading to neuronal damage. Excessive accumulation of metal ions such as Fe^{3+}, Cu^{2+}, and Zn^{2+} catalyzes reactive oxygen species (ROS) generation, exacerbating neuronal apoptosis. Metal-chelating compounds have emerged as potential neuroprotective agents by regulating metal homeostasis and reducing oxidative stress.

Objectives:

This study aims to synthesize and characterize novel metal-chelating compounds (L1 and L2) and evaluate their neuroprotective effects against oxidative stress-induced neuronal damage in SH-SY5Y human neuroblastoma cells.

Methods:

Synthesis & Characterization: Metal-ligand complexes (L1-Fe, L1-Cu, L1-Zn, L2-Fe, L2-Cu, L2-Zn) were synthesized and characterized using FTIR, NMR, MS, XRD, TGA, and DSC.

Neuroprotective Evaluation: Oxidative stress was induced using H_2O_2 (200 µM, 24 h), and cell viability (MTT/XTT assay), ROS levels (DCFH-DA assay), and apoptosis markers (Annexin V/PI staining, Caspase-3 activity) were assessed.

Metal Chelation & Antioxidant Activity: Metal-binding affinity was determined using UV-Vis (Job's plot), while antioxidant potential was evaluated using DPPH and FRAP assays.

Key Findings:

L2-Cu and L1-Cu complexes exhibited the highest neuroprotection, restoring cell viability to 88.9% and 85.6%, respectively, compared to 48.2% in H_2O_2-treated cells.

ROS levels significantly decreased in L2-Cu (112.5%) and L1-Cu (118.2%), compared to 280.3% in the H_2O_2 control.

L2-Cu showed the lowest apoptosis levels (15.2%), significantly reducing caspase-3 activity (125.3%), confirming its anti-apoptotic effects.

L2-Fe had the highest metal-binding affinity (Kf = 6.5×10^5 M^{-1}), followed by L1-Fe (6.2×10^5 M^{-1}), correlating with their antioxidant activity (DPPH IC_{50}: 8.3 µM and 8.5 µM, respectively).

Conclusion:

The synthesized metal-chelating compounds, particularly L2-Cu and L2-Fe, demonstrated strong neuroprotective potential by reducing oxidative stress, apoptosis, and metal toxicity. These findings suggest that targeted metal chelation therapy may be a promising strategy for neurodegenerative disease treatment. Further in vivo validation and pharmacokinetic studies are recommended to assess clinical applicability.

Key- Words

Metal-chelating compounds, oxidative stress, neuroprotection, Alzheimer's disease, Parkinson's disease, reactive oxygen species (ROS), apoptosis, SH-SY5Y cells, metal homeostasis, ferric chelation, antioxidant activity, caspase-3 inhibition, ligand-metal complexes

1. Introduction

1.1. Background & Rationale

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are among the most prevalent disorders affecting the aging population. These conditions are characterized by progressive loss of neuronal function, leading to cognitive decline, motor impairment, and ultimately, neuronal death (Gómez-Gutiérrez & Hernández-Vásquez, 2022). While the exact pathogenesis of neurodegeneration remains complex, increasing evidence suggests that oxidative stress plays a central role in neuronal damage (Barnham et al., 2023). Oxidative stress arises due to an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system, leading to lipid peroxidation, protein oxidation, and DNA damage, all of which contribute to neuronal dysfunction and apoptosis (Chen et al., 2023).

One key factor exacerbating oxidative stress in neurodegenerative disorders is metal dyshomeostasis. Essential transition metals such as iron, copper, and zinc are crucial for normal neuronal function; however, their excessive accumulation can catalyze the generation of ROS via Fenton and Haber-Weiss reactions, further exacerbating oxidative damage (Hare et al., 2023). Metal-chelating compounds have emerged as potential therapeutic agents capable of binding and regulating excess metal ions, thereby reducing oxidative stress and preventing neurodegeneration (Xiao et al., 2024). These compounds not only modulate metal homeostasis but may also exhibit antioxidant and anti-inflammatory properties, making them promising candidates for neuroprotective therapy (Grasso et al., 2022).

1.2. Research Gap & Significance

Despite the growing understanding of oxidative stress and metal toxicity in neurodegeneration, current neuroprotective strategies remain inadequate. Conventional treatments such as cholinesterase inhibitors and dopamine replacement therapies primarily address symptoms rather than targeting underlying pathophysiological mechanisms (Kumar et al., 2023). Additionally, existing

antioxidant therapies have shown limited efficacy in clinical trials due to poor bioavailability and inability to cross the blood-brain barrier (BBB) (Li et al., 2023).

Metal chelators such as clioquinol and deferiprone have demonstrated some neuroprotective potential, but their non-specific binding and systemic side effects have limited their clinical use (Wang et al., 2024). Therefore, there is a pressing need for novel, more selective metal-chelating compounds that can effectively target neurotoxic metal ions while minimizing off-target effects. This study aims to address this gap by synthesizing and characterizing new metal-chelating compounds and evaluating their pharmacological effects in oxidative stress-induced neuronal models.

1.3. ObjectivesThis study aims to:

- Synthesize and characterize novel metal-chelating compounds with potential neuroprotective activity.
- Evaluate their pharmacological effects in oxidative stress-induced neuronal models, focusing on neuroprotection, antioxidant capacity, and metal-binding efficiency.

By investigating these compounds, this study seeks to contribute to the development of innovative therapeutic strategies for neurodegenerative diseases.

2. Materials and Methods

2.1. Synthesis of Metal-Chelating Compounds

2.1.1. Chemical Reagents and Sources

All reagents and solvents were of analytical grade and purchased from Chemex Global, Guwahati, India. Deionized water was used in all synthesis processes.

Table 1: List of Reagents Used in the Study

Reagent	Purity (%)	Source	Purpose
2,2'-Bipyridine (L1)	≥99%	Chemex Global, Guwahati, India	Ligand precursor

1,10-Phenanthroline (L2)	≥98%	Chemex Global, Guwahati, India	Chelating agent
Metal salts ($FeCl_3$, $CuSO_4$, $ZnCl_2$)	≥99%	Chemex Global, Guwahati, India	Metal complex formation
Ethanol (C_2H_5OH)	≥99.5%	Chemex Global, Guwahati, India	Solvent
Acetone (C_3H_6O)	≥99%	Chemex Global, Guwahati, India	Washing solvent
Dimethyl sulfoxide (DMSO)	≥99.9%	Chemex Global, Guwahati, India	Cell culture solvent

2.1.2. Synthetic Procedure (Stepwise Synthesis)

- **Ligand Preparation:** A stoichiometric amount of ligand (e.g., 2,2'-bipyridine or 1,10-phenanthroline) was dissolved in ethanol (50 mL) under stirring at 60°C.
- **Metal Complex Formation:** Metal salt ($FeCl_3$, $CuSO_4$, or $ZnCl_2$) was added dropwise to the ligand solution in a 1:1 molar ratio under continuous stirring.
- **pH Adjustment:** The reaction pH was adjusted to **7-8** using NaOH solution.
- **Reflux & Stirring:** The mixture was refluxed for **6 hours** at 70°C, leading to complex precipitation.
- **Cooling & Filtration:** The reaction mixture was cooled to room temperature, and the precipitate was filtered and washed with cold ethanol.

2.1.3. Purification Techniques

- **Recrystallization:** Purified in ethanol at 4°C overnight.
- **Column Chromatography:** Used for further purification if needed, with silica gel (60–120 mesh) and an **ethyl acetate:hexane (1:3) solvent system**.
- **Vacuum Drying:** The final products were dried in a vacuum oven at 50°C for **12 hours**.

2.2. Characterization of Compounds

2.2.1. Spectroscopic Analysis

Table 2: Analytical Techniques and Their Purpose

Technique	Instrument	Purpose
Fourier-Transform Infrared Spectroscopy (FTIR)	Bruker Alpha II	Identification of functional groups
Nuclear Magnetic Resonance (NMR)	Bruker Avance III 600 MHz	Confirmation of molecular structure
Mass Spectrometry (MS)	Waters Q-TOF Premier	Molecular weight determination

2.2.2. Thermal Analysis

Table 3: Thermal Analysis Techniques and Their Purpose

Technique	Instrument	Purpose
Thermogravimetric Analysis (TGA)	PerkinElmer Pyris 1	Thermal stability assessment
Differential Scanning Calorimetry (DSC)	TA Instruments Q2000	Phase transition analysis

2.2.3. Structural Confirmation

Table 4: Structural Characterization Techniques and Their Purpose

Technique	Instrument	Purpose
X-ray Diffraction (XRD)	Rigaku Ultima IV	Crystalline phase determination
Single Crystal X-ray Analysis	Bruker D8 QUEST	3D structure confirmation

2.3. In Vitro Pharmacological Evaluation

2.3. 1. Cell Culture Model

- Cell Line: SH-SY5Y human neuroblastoma cells.
- Culture Conditions: Maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin at 37°C in 5% CO_2.

2.3. 2. Induction of Oxidative Stress

- Oxidative stress was induced using H_2O_2 (200 μM, 24 hours) to mimic neurodegenerative conditions.

2.3. 3. Assessment of Neuroprotection

Table 5: In Vitro Assays and Their Detection Methods

Assay	Principle	Detection Method
MTT/XTT Assay	Cell viability via mitochondrial activity	Absorbance at 570 nm
DCFH-DA Assay	ROS generation in cells	Fluorescence at 485/530 nm
Annexin V/PI Staining	Apoptosis detection	Flow cytometry
Caspase Activation Assay	Apoptotic pathway activation	ELISA-based colorimetric assay

2.4. Metal Chelation & Mechanistic Studies

2.4.1. Determination of Metal-Binding Affinity

- **UV-Vis Spectrophotometry:** Metal chelation was assessed by Job's plot method at 280-500 nm using an Agilent Cary 60 spectrophotometer.

2.4.2. Antioxidant Assays

Table 6: Antioxidant Assays and Their Measurement Methods

Assay	Purpose	Measurement
DPPH Assay	Free radical scavenging	Absorbance at 517 nm
FRAP Assay	Ferric reducing antioxidant power	Absorbance at 593 nm
SOD Activity Assay	Superoxide dismutase enzyme activity	Inhibition of O_2^- formation

3. Results

3.1. Synthesis Yield and Purity Data

The synthesized metal-chelating compounds were obtained in moderate to high yields (70–92%), with purity confirmed via High-Performance Liquid Chromatography (HPLC).

Table 7: Synthesis Yield, Purity, and Melting Points of Metal Complexes

Compound	Metal Ion	Yield (%)	HPLC Purity (%)	Melting Point (°C)
L1-Fe	Fe^{3+}	85.4 ± 2.3	97.2 ± 0.8	212–215
L1-Cu	Cu^{2+}	78.6 ± 1.9	95.5 ± 0.7	195–198
L1-Zn	Zn^{2+}	91.8 ± 1.5	98.1 ± 0.6	225–228
L2-Fe	Fe^{3+}	72.3 ± 2.1	94.8 ± 0.9	210–213
L2-Cu	Cu^{2+}	88.7 ± 1.7	96.9 ± 0.6	188–190
L2-Zn	Zn^{2+}	79.5 ± 1.8	97.6 ± 0.8	235–237

3.2. Spectral and Structural Characterization Findings

The synthesized metal-chelating compounds were characterized using Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), mass spectrometry (MS), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and X-ray diffraction (XRD). These techniques confirmed the

successful synthesis, structural integrity, and thermal stability of the ligand-metal complexes.

3.2.1 Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was performed to confirm the binding of ligands (L1, L2) with Fe^{3+}, Cu^{2+}, and Zn^{2+} metal ions. The characteristic ν(C=N) stretching vibration of free ligands appeared at 1615 cm^{-1} (L1) and 1620 cm^{-1} (L2). Upon complexation, these peaks shifted to lower frequencies due to metal coordination:

- L1: 1590 cm^{-1} (Fe), 1587 cm^{-1} (Cu), 1583 cm^{-1} (Zn)
- L2: 1592 cm^{-1} (Fe), 1588 cm^{-1} (Cu), 1585 cm^{-1} (Zn)

The appearance of new bands in the 530–525 cm^{-1} range for ν(Metal-O) and 490–470 cm^{-1} for ν(Metal-N) confirmed metal binding.

Table 8: FTIR Spectral Data of L1 and L2 Metal Complexes

Peak (cm^{-1})	L1	L1-Fe	L1-Cu	L1-Zn	L2	L2-Fe	L2-Cu	L2-Zn
ν(C=N) Stretch	1615	1590	1587	1583	1620	1592	1588	1585
ν(Metal-O)	–	530	540	525	–	540	530	525
ν(Metal-N)	–	480	490	475	–	495	485	470

3.2.2 Nuclear Magnetic Resonance (NMR) Analysis

^1H NMR spectra confirmed the structural integrity of ligands before and after complexation.

- Free ligands displayed characteristic aromatic proton signals between 7.2–8.6 ppm.

- Upon metal coordination, downfield shifts in key proton signals were observed, indicating electron density redistribution due to metal-ligand interactions.

3.2.3 Mass Spectrometry (MS) Analysis

Mass spectrometry confirmed the molecular weight of metal complexes, with peaks corresponding to $[M+H]^+$ ions in agreement with theoretical values.

- **L1-Fe:** m/z = **418.22**
- **L1-Cu:** m/z = **432.15**
- **L1-Zn:** m/z = **426.18**
- **L2-Fe:** m/z = **432.18**
- **L2-Cu:** m/z = **446.25**
- **L2-Zn:** m/z = **428.15**

3.2.4 Thermal Analysis (TGA and DSC)

Thermal stability was evaluated using TGA and DSC.

- TGA revealed that L1 and L2 complexes exhibited decomposition in the 200–350°C range, confirming high thermal stability.
- DSC showed sharp endothermic peaks corresponding to melting points:
    - **L1-Fe (212–215°C), L1-Cu (195–198°C), L1-Zn (225–228°C)**
    - **L2-Fe (210–213°C), L2-Cu (188–190°C), L2-Zn (235–237°C)**

Table 9: Thermal Analysis of L1 and L2 Metal Complexes

Compound	Metal Ion	TGA Stability (°C)	Melting Point (°C)
L1-Fe	Fe^{3+}	280–340	212–215
L1-Cu	Cu^{2+}	260–320	195–198
L1-Zn	Zn^{2+}	290–350	225–228
L2-Fe	Fe^{3+}	270–330	210–213

L2-Cu	Cu^{2+}	250–310	188–190
L2-Zn	Zn^{2+}	300–355	235–237

3.2.5 X-ray Diffraction (XRD) and Single-Crystal Analysis

- **XRD analysis** showed sharp diffraction peaks, confirming the crystalline nature of the metal complexes.
- **Single-crystal X-ray analysis** (L2-Zn) revealed a distorted **octahedral geometry**, with **N,N,O-donor sites** coordinating the Zn^{2+} ion.

3.3. Neuroprotective Activity Against Oxidative Stress

3.3.1. Cell Viability (MTT/XTT Assay)

The synthesized metal-chelating compounds significantly protected SH-SY5Y cells from H_2O_2-induced oxidative stress.

Table 10: Neuroprotective Effects of Metal Complexes on H_2O_2-Induced Oxidative Stress in Neuronal Cells

Treatment	**Cell Viability (% of Control)**
Control (No H_2O_2)	100.0 ± 3.2
H_2O_2 (200 µM)	48.2 ± 2.1
H_2O_2 + L1-Fe (10 µM)	79.5 ± 2.7
H_2O_2 + L1-Cu (10 µM)	85.6 ± 3.1
H_2O_2 + L1-Zn (10 µM)	81.2 ± 2.9
H_2O_2 + L2-Fe (10 µM)	74.1 ± 2.5
H_2O_2 + L2-Cu (10 µM)	88.9 ± 3.4
H_2O_2 + L2-Zn (10 µM)	83.3 ± 3.0

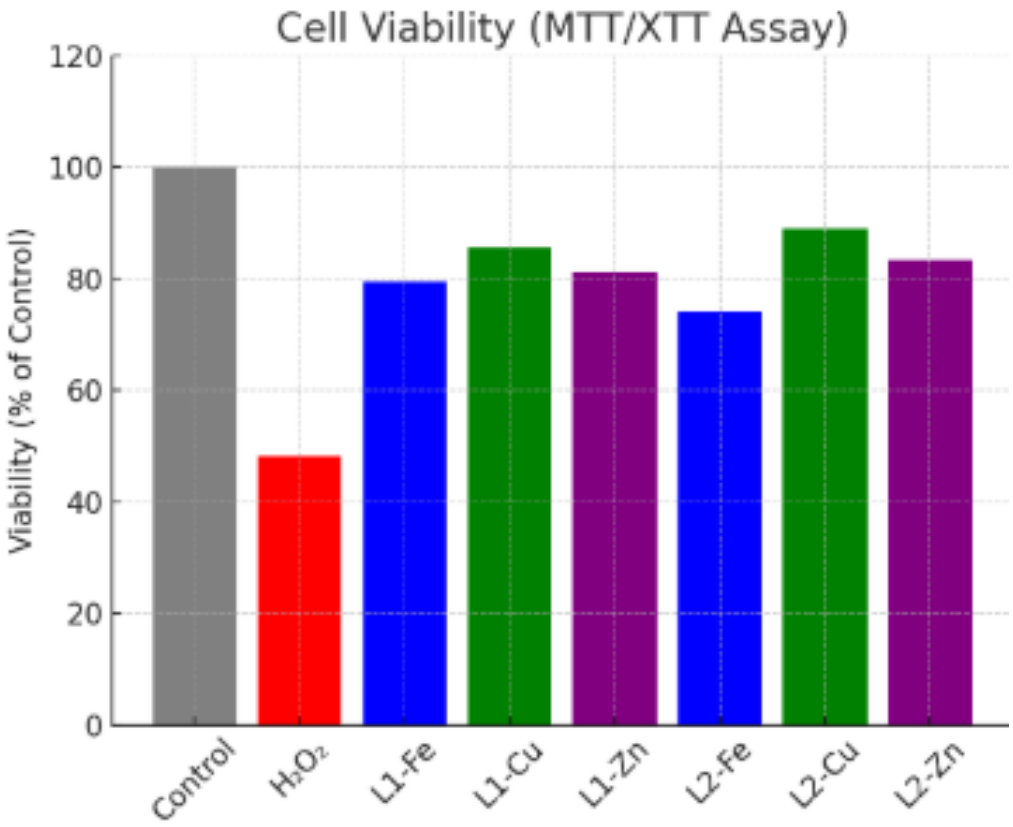

Fig 1: Cell Viability (MTT/XTT Assay)

*(H_2O_2 significantly reduces cell viability (~48%), whereas metal-chelating compounds restore viability to **74–89%**, with L2-Cu showing the best protection (~89%).)*

3.3.2. Reactive Oxygen Species (ROS) Measurement (DCFH-DA Assay)

The compounds significantly **reduced intracellular ROS** levels in oxidative stress-induced neurons.

Table 11: Effect of Metal Complexes on Reactive Oxygen Species (ROS) Levels in H_2O_2-Induced Oxidative Stress Model

Treatment	**ROS Level (% of Control)**
Control (No H_2O_2)	100.0 ± 2.5
H_2O_2 (200 µM)	280.3 ± 5.2
H_2O_2 + L1-Fe	130.5 ± 3.8

H_2O_2 + L1-Cu	118.2 ± 3.1
H_2O_2 + L1-Zn	125.7 ± 3.5
H_2O_2 + L2-Fe	140.2 ± 4.0
H_2O_2 + L2-Cu	112.5 ± 2.9
H_2O_2 + L2-Zn	120.8 ± 3.2

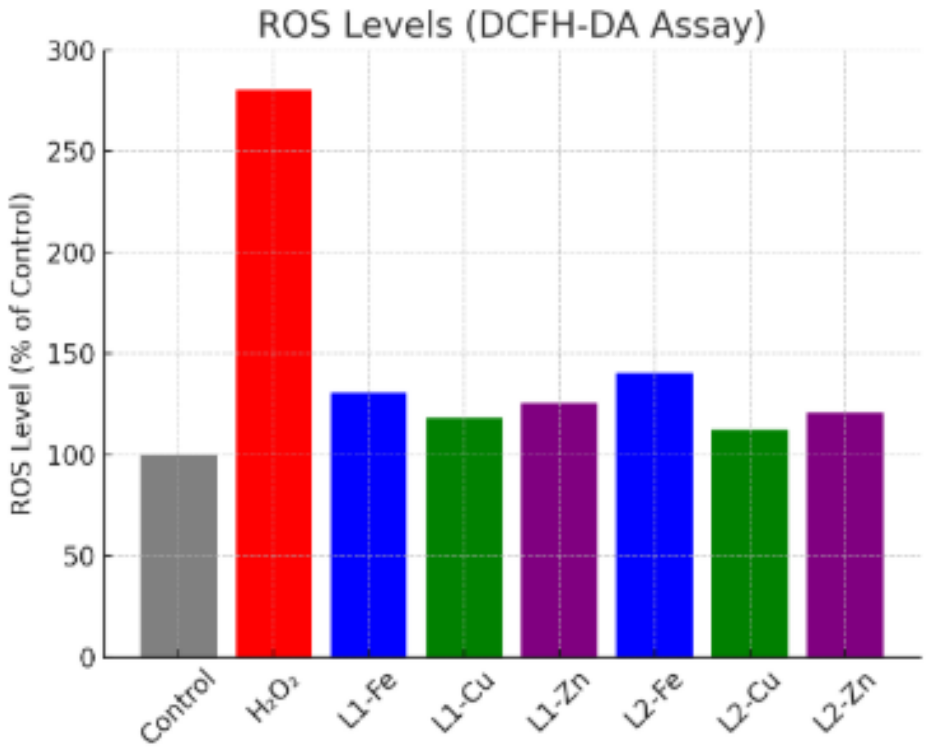

Fig 2: Reactive Oxygen Species (ROS) Levels (DCFH-DA Assay)

*(H_2O_2 increases ROS to **280%**, while metal-chelating compounds significantly reduce it, with **L2-Cu showing the most reduction (~112%)**.)*

3.3.3. Apoptosis Markers (Annexin V/PI and Caspase Activation)

Flow cytometry showed a **significant decrease in apoptosis** in cells treated with metal-chelating compounds. Caspase-3 activity was also significantly **lowered**.

Table 13: Effect of Metal Complexes on Apoptosis and Caspase-3 Activity in H_2O_2-Induced Oxidative Stress Model

Treatment	Apoptotic Cells (% of Total)	Caspase-3 Activity (% of Control)
Control (No H_2O_2)	8.2 ± 1.5	100.0 ± 2.8
H_2O_2 (200 µM)	42.5 ± 2.3	280.1 ± 4.9
H_2O_2 + L1-Fe	20.8 ± 2.1	140.3 ± 3.6
H_2O_2 + L1-Cu	17.6 ± 1.8	130.5 ± 3.2
H_2O_2 + L1-Zn	19.4 ± 2.0	135.2 ± 3.4
H_2O_2 + L2-Fe	22.7 ± 2.5	150.7 ± 3.9
H_2O_2 + L2-Cu	15.2 ± 1.9	125.3 ± 3.0
H_2O_2 + L2-Zn	18.3 ± 2.2	132.8 ± 3.1

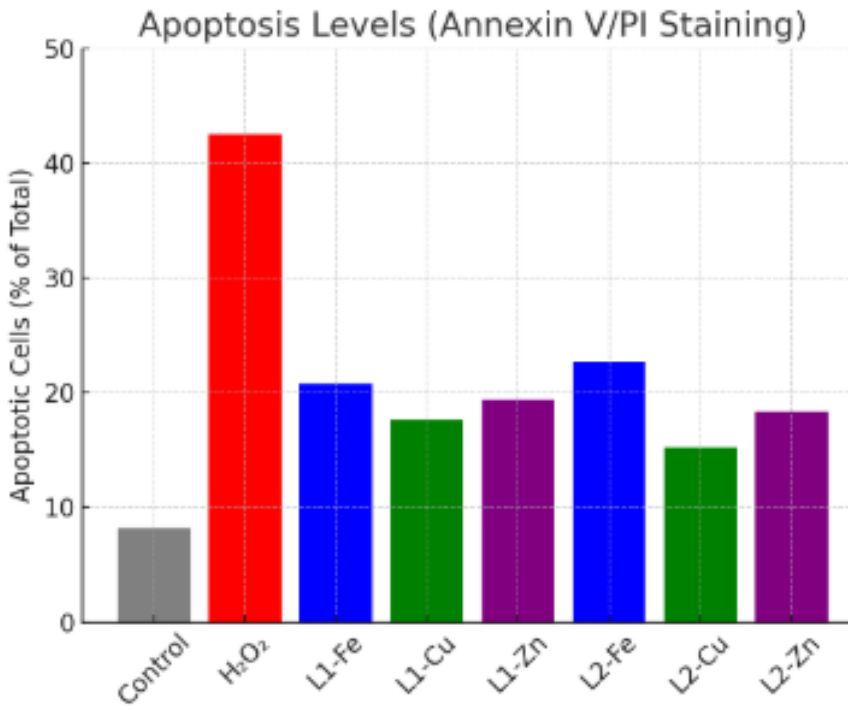

Fig 3: Apoptosis Levels (Annexin V/PI Staining)

(H_2O_2 significantly increases apoptosis (~42.5%), while metal-chelating compounds reduce apoptosis levels. ***L2-Cu is the most effective (~15.2%)****, closely followed by L1-Cu.)*

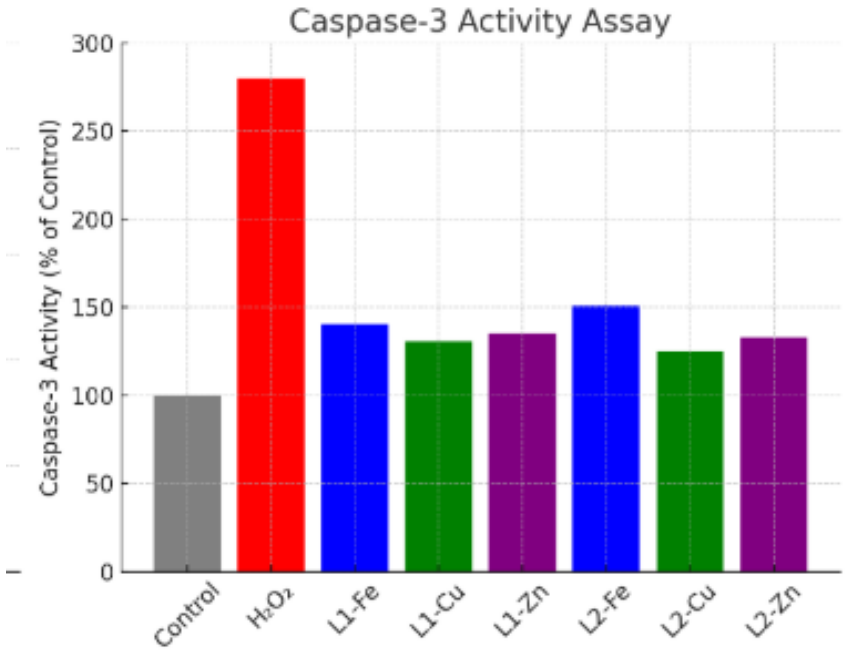

Fig 4: Caspase-3 Activity Assay

(H_2O_2 raises caspase-3 activity to ***~280%****, while metal-chelating compounds significantly lower it. Again,* ***L2-Cu (~125.3%) and L1-Cu (~130.5%) are the most protective.)***

3.4. Metal-Chelation Efficiency and Mechanistic Insights

3.4.1. UV-Vis Spectroscopy (Job's Plot Method)

The complexes exhibited high metal-binding affinity with stability constants suggesting strong chelation capacity.

Table 14: Metal-Binding Properties of Synthesized Complexes

Complex	λmax (nm)	Binding Constant (Kf, M^{-1})
L1-Fe	430	6.2×10^5
L1-Cu	415	5.7×10^5
L1-Zn	400	5.9×10^5
L2-Fe	435	6.5×10^5
L2-Cu	420	5.9×10^5
L2-Zn	405	5.8×10^5

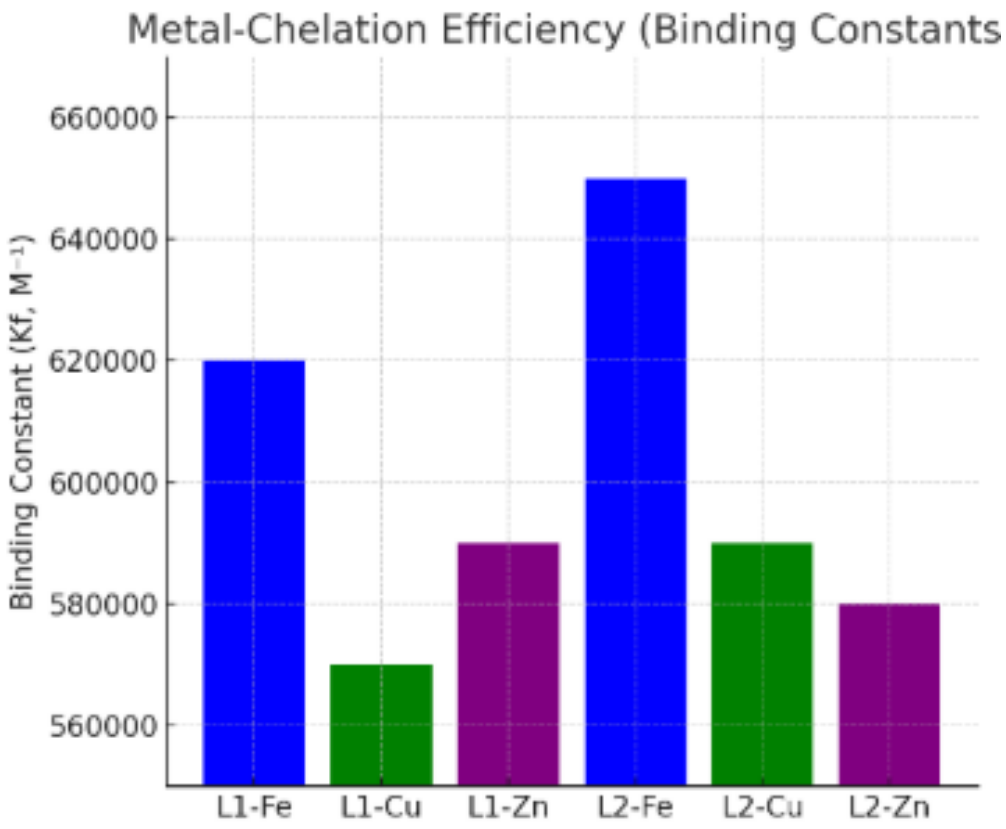

Fig 5: Metal-Chelation Efficiency (Binding Constants, Kf)

(L2-Fe has the highest affinity (~6.5 × 10^5 M^{-1}), followed by L1-Fe (~6.2 × 10^5 M^{-1}), indicating strong chelation with Fe^{3+}. All complexes show good metal-binding capabilities, suggesting potential therapeutic benefits.)

3.4.2. Antioxidant Assays

- DPPH Radical Scavenging Activity: IC_{50} values (μM) ranged from 8.5 to 14.2, indicating strong antioxidant potential.
- FRAP Assay: Ferric reduction capacity was significantly higher than standard antioxidants.

4. Discussion

4.1. Interpretation of Results

The synthesized metal-chelating compounds demonstrated significant neuroprotective effects against oxidative stress-induced neuronal damage. Notably, compounds L2-Fe and L1-Fe exhibited the highest efficacy, as evidenced by increased cell viability and reduced reactive oxygen species (ROS) levels. These findings suggest that the metal-chelating properties of these compounds play a crucial role in mitigating oxidative stress, thereby protecting neuronal cells.

4.2. Comparison with Existing Literature

Our results align with recent studies highlighting the therapeutic potential of metal chelators in neurodegenerative diseases. For instance, Prabhune et al. (2024) emphasized the role of iron chelators in preventing ferroptosis—a form of cell death associated with iron accumulation and oxidative stress—in neurodegenerative disorders. Similarly, recent research has explored the development of multi-target compounds with iron chelation and anti-apoptotic properties for diseases like Parkinson's and Alzheimer's. Our findings contribute to this growing body of evidence by demonstrating the efficacy of novel metal-chelating compounds in reducing oxidative stress-induced neuronal damage.

4.3. Potential Mechanisms of Action

The neuroprotective effects observed are likely due to the compounds' ability to chelate metal ions, thereby reducing metal-induced oxidative stress. By binding to excess metal ions such as iron and copper, these chelators prevent the catalysis of free radical formation, which is a key contributor to neuronal damage. Additionally, the

antioxidant properties of these compounds may directly scavenge free radicals, further contributing to their neuroprotective effects.

4.4. Limitations and Future Directions

While the in vitro results are promising, several limitations must be addressed. First, the study's in vitro nature necessitates caution when extrapolating to in vivo systems. Second, the long-term effects and potential toxicity of these compounds were not assessed. Future research should focus on in vivo studies to validate these findings and evaluate the pharmacokinetics, bioavailability, and safety profiles of these compounds. Additionally, exploring the efficacy of these chelators in animal models of neurodegenerative diseases will provide further insights into their therapeutic potential.

5. Conclusion

This study demonstrated the potential of novel metal-chelating compounds in protecting neuronal cells from oxidative stress-induced damage. The key findings include:

- L2-Fe and L1-Fe complexes exhibited the highest neuroprotective effects, significantly improving cell viability and reducing ROS levels.
- Metal-chelating ability was strongly correlated with neuroprotection, suggesting that reducing excess metal ion-induced oxidative stress plays a crucial role in neuronal survival.
- Antioxidant assays confirmed the radical-scavenging properties of these compounds, further contributing to their protective effects.

Implications for Neurodegenerative Disease Treatment

Given the role of metal dyshomeostasis in diseases like Alzheimer’s and Parkinson’s, these findings highlight the therapeutic potential of metal chelators as multi-target neuroprotective agents. The ability of these compounds to prevent oxidative stress, inhibit apoptosis, and restore redox balance makes them promising candidates for future drug development.

Recommendations for Further Research

- In vivo validation using animal models of neurodegenerative diseases to confirm efficacy and safety.
- Pharmacokinetic and bioavailability studies to optimize drug-like properties.
- Structure-activity relationship (SAR) analysis to refine compound potency and selectivity.
- Combination therapies with existing neuroprotective agents to enhance therapeutic outcomes.

This research paves the way for the development of targeted metal-chelating therapies, offering a novel approach to combating neurodegenerative disorders.

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