Al-Nisour Journal for Medical Sciences

Manuscript 1131

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AL-NISOUR JOURNAL FOR MEDICAL SCIENCE 2025;7:1–13

Synthesis, Structural Characterization, and In-vitro Cytotoxicity of Zinc-levofloxacin Ligand

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Abstract

Background: This study aimed to modify levofloxacin and evaluate its cytotoxicity as well as the synthesis, characterization and physical evaluation of mixed ligand zinc complex with the third generation quinolones' representative, levofloxacin. Levofloxacin coupled to zinc through one pyridone and one carboxylate oxygen as well as with two nitrogen atoms from the heteroligand, as demonstrated by the complexation of zinc (II) metal ion with the deprotonated ligand and heteroligand. The identification of the complex structure was carried out using Fourier Transform Infrared Spectroscopy, Hydrogen-Nuclear Magnetic Resonance, melting point, and UV–visible spectrophotometry.

Results: Binding of the zinc on the two donor −N− atoms of 1,10-phenanthroline, the obtained ligand was pale yellow powder with melting point, 260–263 °C. The Fourier Transform Infrared spectra, ν(C=O) is responsible for the strong band located at 1722.43 cm^{-1} in the levofloxacin spectrum. The ligand spectra lack this peak, and its place is occupied by strong distinctive bands located at 1718.58 cm^{-1} and 1620.21 cm^{-1}. Concerning UV-visible spectra, the spectra of levofloxacin and the ligand are the same, with a few little variations. Hydrogen-Nuclear Magnetic Resonance spectra of the ligand showed broader signals. The majority of the hydrogen atoms in the levofloxacin molecule exhibit an obvious shift to the downfield region in the zinc complex spectrum. The cytotoxic behavior of the synthesized mixed complex was performed by methylthiotetrazolium assay. It was discovered that following treatment with the compound, HRT-18 cell viability and proliferation rates dropped. The 67-year-old male patient's large intestine included colorectal cancer cells known as HRT-18 cells, which were obtained from his adenocarcinoma.

Conclusions: Through in vitro research, we discovered that complex at 1000 and 500 µg/ml significantly cytotoxically affected the HRT-18 colorectal cancer cell line. The study concluded that the synthesized mixed complex (Levofloxacin coupled to zinc) is a potential cytotoxic agent against HRT-18 colorectal cancer cell line.

Keywords: Levofloxacin coupled to zinc, Cytotoxicity, FT-IR, ^{1}HNMR, HRT-18 colorectal cancer cell

1. Background

Levofloxacin (H-levo) the active isomer of ofloxacin, is a third-generation quinolone antimicrobial agent that exhibits enhanced activity against Gram-positive and atypical organisms while maintaining a broad spectrum of antibacterial activity comparable to that of previous quinolones (Foroumadi *et al.*, 2007). Levofloxacin is indicated for a wide range of infections, including those of the urinary tract, prostatitis, lungs, skin, bones, sinuses, and ears caused by susceptible bacteria, as well as infectious diarrhea brought on by Escherichia coli, Campylobacter jejuni, and Shigella bacteria (Tarushi *et al.*, 2011). Levofloxacin can suppress the early stage of colorectal cancer by decreasing the number and size of ACF and decrease proliferative cell nuclear antigen (PCNA) induced by azoxymethane (AOM) in the colon and rectum of mice (Eman, 2019). Song and colleagues (2016) found that Levofloxacin at (conc. 50–200 µg/ml) for lung cancer treatment that inhibiting proliferation and inducing apoptosis of lung cancer cells by inducing mitochondrial dysfunction and oxidative damage.

Cancer is a type of disease characterized by uncontrolled cell proliferation, there are over a hundred different types of cancer, and each is classified by the type of cell that is initially affected (Howlader *et al.*,

Received 21 September 2024; accepted 3 November 2024.
Available online 9 May 2025

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https://doi.org/10.70492/2664-0554.1131

2017). In general, carcinogenic chemicals, viruses, radiation, and many environmental factors contribute to the initiation of cancer (Waly *et al.*, 2014). Colorectal cancer is among the leading cause of mortality and the incidence rate worldwide (Favoriti *et al.*, 2016). It is ranked as the third-most prevalent malignancy and the fourth leading reason for cancer-related fatalities worldwide, estimating approximately 1,400,000 newly diagnosed cases. And there are roughly 700,000 fatalities worldwide (Glynne-Jones *et al.*, 2017).

Colorectal cancer is a prevalent cancer and one of the main causes of cancer mortality entire the world. Several factors from genetics to diet are involved in the incidence of this malignancy (Khayoon & Al-Rekabi, 2020). Some studies of the Western population have shown that at least 50% of them develop colon and rectal cancer at the age of 70, and transformation into malignancy ensures that, as a result, the second leading cause of cancer-related deaths in the United States, after lung cancer, is colorectal cancer when cancer caused by smoking is not included (Rawla *et al.*, 2019).

One of the most difficult illnesses to treat, colorectal cancer has severe symptoms that worsen, become more noticeable, and are particular as the disease advances. It has a high fatality rate and a very short survival time if detected too late or if treatment is not received (Mahmood *et al.*, 2017). Fresh bleeding was the most frequently reported symptom, although weight loss was the least prevalent. The rectum was the most frequently found site of colorectal cancer, followed by the sigmoid colon, and the cecum, while the least frequently found single site was the ascending colon. Rectum and sigmoid area tumors were found in 77.8% of cases of colorectal cancer, a significantly higher frequency than tumors in other colonic sites (Alrubaie *et al.*, 2019). The most common tumor found in the colon and rectum is called an adenocarcinoma, and it is also the most commonly identified type of colorectal carcinoma. The anatomical and developmental origin of the tumors, unique carcinogenic factors (e.g., various bacterial populations on opposite sides of the colon, being exposed to specific nutrients and bile acids), or a number of factors can be responsible for the variation in tumor sites (Falih Soliman & Jasim Mohamad, 2022).

Cancer drug resistance is a complex phenomenon that is influenced by drug inactivation, drug target alteration, drug efflux, DNA damage repair, cell death inhibition, inherent cell heterogeneity, epigenetic effects, or any combination of these mechanisms (Housman *et al.*, 2014).

Chemotherapy drugs are powerful enough to kill rapidly growing cancer cells, they can also harm healthy cells. This may cause a variety of side effects. The severity of these side effects depends on the stage of cancer and the type and amount of chemotherapy (Nurgali *et al.*, 2018). The nature of the peripheral substituents and the bicyclic heteroaromatic pharmacophore of fluoroquinolones, such levofloxacin, determine their antibacterial activity. Individuals with *P. aeruginosa* infections frequently receive therapy with fluoroquinolones, especially ciprofloxacin and levofloxacin (Abdullah & Abdulkareem, 2009). More precisely, fluoroquinolones change the relative pharmacokinetics while increasing affinity for bacterial enzymes and enhancing cell penetration. Furthermore, fluoroquinolone metal complexes have been extensively investigated for their ability to interact with DNA in addition to their antibacterial activity against a variety of microorganisms, demonstrating the significance of metal ions in the mechanism of action of these medications (Tarab, 2022; Katsarou *et al.*, 2008).

1, 10-Phenanthroline functions as a potent double-stranded DNA binder and makes it easier for the hydrogen atom to be extracted from the sugar unit, making it a desirable ligand. According to Katsarou *et al.* (2008) and Chen *et al.* (2013), metal complexes of 1, 10-phenanthroline, for example, have intriguing anti-cancer characteristics. Furthermore, 1,10-phenanthroline complexes and their derivatives are recognized to be extremely significant due to their wide range of biological activities, including antibacterial and anticancer properties (Katsarou *et al.*, 2008).

Since chemotherapy medications can have a wide range of side effects and cancers might become resistant to current therapies, further research and treatment development are required to tackle such problems and due to the success of levofloxacin and levofloxacin-metal-based combinations in several experimental in *vitro* cancer studies, the present study aimed to synthesize of zinc-levofloxacin ligand with the modified method and to investigate the potential anti-colorectal cancer activity of zinc-levofloxacin ligand in *vitro* against the HRT-18 colorectal cancer cell line.

2. Methods

2.1. Reagents and Materials

For zinc-levofloxacin ligand synthesis, analytical-grade levofloxacin (Sigma, UK), $ZnCl_2$ (Sigma, UK), methanol (Merck, Germany), 1,10-phenanthroline (Kanto chemical, Japan) and solvents were utilized. Levofloxacin and zinc chloride from Sigma were utilized without additional purification. The methanol was used exactly as it was received from Merck.

2.2. Synthesis and characterization of zinc-levofloxacin ligand

2.2.1. Synthesis of zinc-levofloxacin ligand

A solution of $ZnCl_2$ (38 mg) in methanol (10 ml) was added to a solution of 1,10-phenanthroline (55 mg) in methanol (5 ml) after an already-prepared solution of levofloxacin (100 mg) in methanol (5 ml) had been added. After 30 minutes of heating and stirring, the resultant liquid was concentrated to half of its initial volume. Next, the building complex was left overnight at room temperature.

A fine, yellow, amorphous substance was generated the next day. After filtering it out, cold methanol was used to wash it (in an ice-filled container). After being well crushed with a clean spatula, the dry precipitate is a fine, yellow, amorphous powder that can be weighed. The approach used here was modified from the (Galani *et al.*, 2014).

2.3. Evaluation of zinc-levofloxacin ligand

The generated complex was investigated according to the following physicochemical and analytical criteria:

2.3.1. Melting point

It is recommended to put the complex in a capillary tube with a closed end and place it inside a melting point reader (Electrothermal, UK). Find out what temperature causes the combination to become liquid.

2.4. Fourier Transform Infrared Spectroscopy (FT-IR)

The organic pollutants detection laboratory/food contamination research center/department of Environment, water and renewable energy/Ministry of Sciences and Technology did perform FT-IR. For all of the many kinds of materials, FT-IR (Shimadzu FT-IR-8400, Japan) is the infrared spectroscopy identification (qualitative analysis) technique that is most frequently utilized (Sousa *et al.*, 2012).

2.5. Hydrogen-Nuclear Magnetic Resonance (^{1}H-NMR)

The ^{1}H-NMR was determined at the Sharif University of Technology's BPC Analysis Center in Tehran, Iran. To determine a molecule's chemical molecular structure, hydrogen-NMR spectroscopy (BRUKER-VARIAN INOVA, USA) was used. ^{1}H-NMR was performed using NMR to collect both quantitative and qualitative information about the composition of a sample. The item under investigation is put into the NMR apparatus, which is encircled by a magnetic field, after being dissolved in a liquid. The NMR spectrum shows the chemical shifts of individual nuclei and is frequently used to ascertain the structure of compounds. With a temperature range of 24 °C to 129 °C, the hydrogen NMR is a useful tool for both low-temperature chemical reaction monitoring and high-temperature analysis of polymers and other materials.

2.6. UV–visible spectrophotometry

This investigation was carried out in the organic pollutants detection laboratory/food contamination research center/department of Environment, water and renewable energy of the Ministry of Sciences and Technology. Methanol (5×10^{-3} M) was used to prepare stock solutions of levofloxacin and zinc-levofloxacin ligand. In three to four hours, solution spectra (UV-1600 series–spectrophotometer, Japan) were obtained. The mixture was exposed to light and allowed to come to room temperature. Additionally, the solution was exposed to UV radiation (200–800 nm) for 30 minutes.

2.7. In vitro cytotoxicity assay

An experiment was carried out to examine the cytotoxicity of zinc-levofloxacin ligand utilizing a colorectal cancer cell line. The 67-year-old male patient's large intestine included colorectal cancer cells known as HRT-18 cells, which were obtained from his adenocarcinoma. The Iraqi Center for Cancer and Medical Genetic Research (ICCMGR) at Al-Mustansiriyah University provided the patient's cells. These cells were used in toxicological and oncological research. To reap the benefits, these cells can undergo high-rate examination.

2.8. Maintenance of cell cultures

This experiment was carried out at the Iraq Biotech facility, which is situated on Al-Harithia Street in Baghdad. Minimum Essential Medium (MEM) (Capricorn, Germany) supplemented with 10% fetal bovine, 100 units /mL penicillin, and 100 µg/mL streptomycin was used to cultivate HRT18 cell lines. After being passed through a solution containing trypsin and EDTA, the cells were maintained at 37 °C and reseeded twice a week at 50% confluence (Attoub *et al.*, 2018).

2.9. Cytotoxicity Assays

The Methylthiotetrazolium (MTT) cell viability experiment was performed on 96-well plates to ascertain the cytotoxic effect (Al-Shammari *et al.*, 2015). Cell

lines were seeded at 1×10^4 cells/well. After a day or until a confluent monolayer was achieved, cells were exposed to varying quantities of zinc-levofloxacin ligand at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml. After exposing the cells to a 2 mg/mL methylthiotetrazolium solution (Bio-World, USA) for 72 hours, the medium was taken out, 28 µL of the solution was added, and the cells were incubated for 1.5 hours at 37 °C to assess their viability. After the MTT solution was removed, 130 µl of dimethyl sulphoxide (DMSO) (Santacruz Biotechnology, USA), was added to each well in order to dissolve any remaining crystals. Shaking the liquid at 37 °C for 15 minutes was part of this process (Adil *et al.*, 2020). At the test wavelength of 492 nm, the assay was performed in triplicate, and the absorbency was determined using a microplate reader. The percentage of cytotoxicity, or the rate at which cell growth is inhibited, was calculated using the following formula (Abdullah *et al.*, 2020):

$$\text{Inhibition rate (IR)} = A - B/A * 100$$

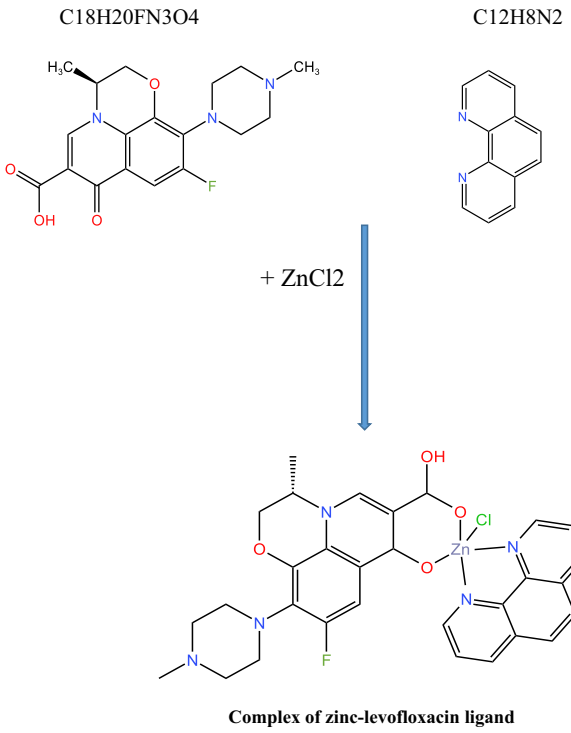
where A is the optical density of control, and B is the optical density of the zinc-levofloxacin (ligand).

% cell viability = (absorbance of treated cell / absorbance of non-treated cell) × 100
% cytotoxicity = 100 − cell viability

3. Results

3.1. Synthesis

Zinc-levofloxacin ligand has been synthesized via the reaction of equimolar amounts of zinc chloride, levofloxacin, and heteroligand 1,10-phenanthroline were used to create the complex, which produced a yellow powder solid that was soluble in methanol. Zinc complexes' interaction with deprotonated levofloxacin demonstrates that levofloxacin coupled to zinc metallic ion via pyridone, one carboxylate oxygen, and two nitrogen atoms from the 1,10-phenanthroline hetero-ligand (Scheme 1 and Fig. 1).



Scheme 1. Synthesis of zinc-levofloxacin ligand.

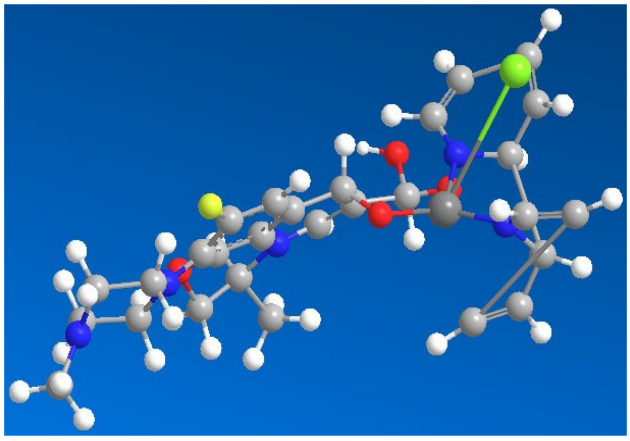


Fig. 1. 3D structure of zinc-levofloxacin ligand (Galani et al., 2014).

3.2. Structural Characterization of zinc-levofloxacin ligand

3.2.1. FT-IR

Concerning the FT-IR spectra, v(C=O) is responsible for the strong band located at 1722.43 cm^{-1} in the levofloxacin spectrum. The complex spectra lack this peak, and its place is occupied by strong distinctive bands located at 1718.58 cm^{-1} and 1620.21 cm^{-1}. between 1800 and 1100 cm^{-1} is the most prominent part of the levofloxacin FTIR spectrum. The carbonyl group's strong absorption band can be seen at 1722.43 cm^{-1}, whereas the pyridone stretch v(c=o) may be found at 1620.21 cm^{-1}, 1533.41 cm^{-1}, and 1456.26 cm^{-1} (Figs. 2 and 3).

3.2.2. UV–visible spectrophotometry

To examine the optical characteristics of the produced samples, a UV-visible quartz cuvette with a one-centimeter path length was utilized. The association between wavelength and absorbance for

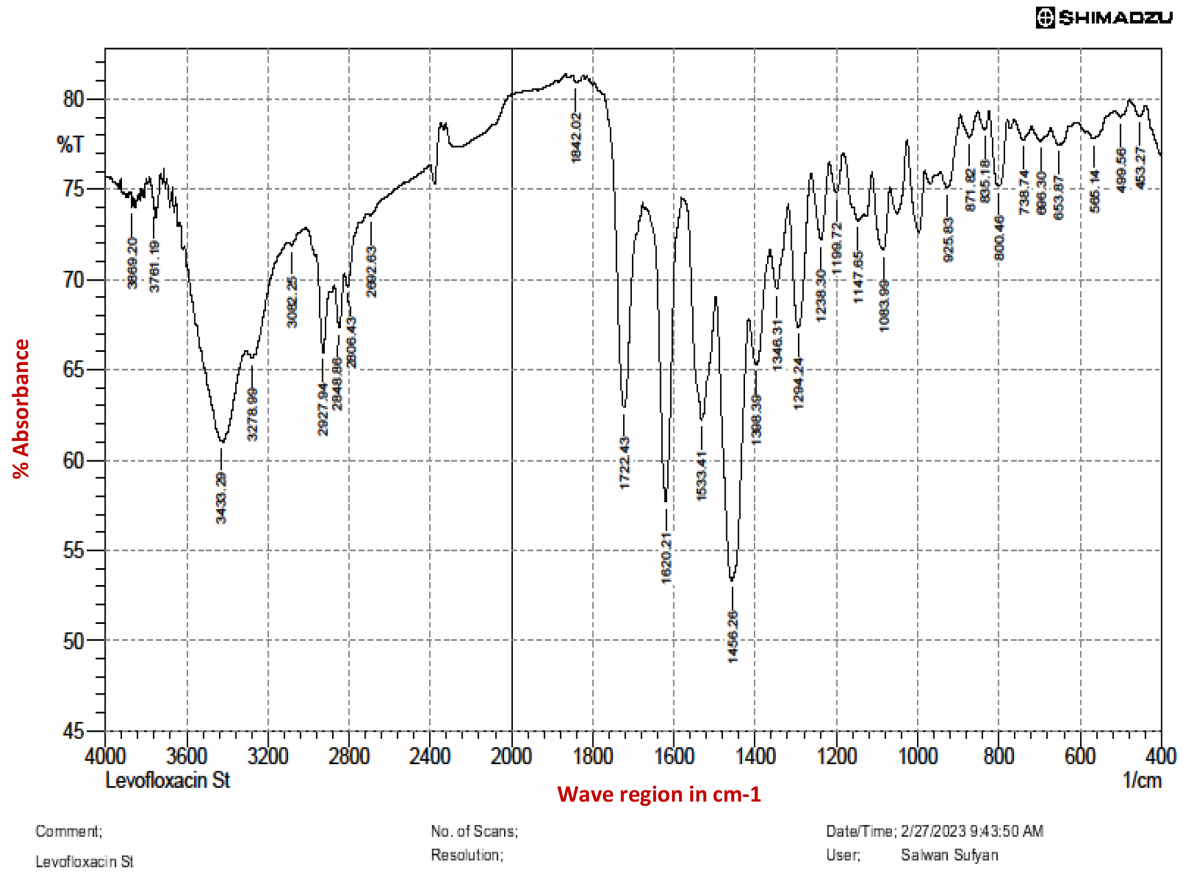


Fig. 2. FT-IR spectra (1100–1800 cm−1 region) of levofloxacin.

both zinc-levofloxacin ligand and pure levofloxacin is depicted in Figs. 4 and 5. Based on the figures, the levofloxacin absorption seen in the wavelength range of 200–400 nm, and show that the samples have a high absorption capacity for electromagnetic radiation in the ultraviolet area, which is consistent with levofloxacin's optical behavior. With the exception of minor changes, the spectra of zinc-levofloxacin ligand and levofloxacin are identical.

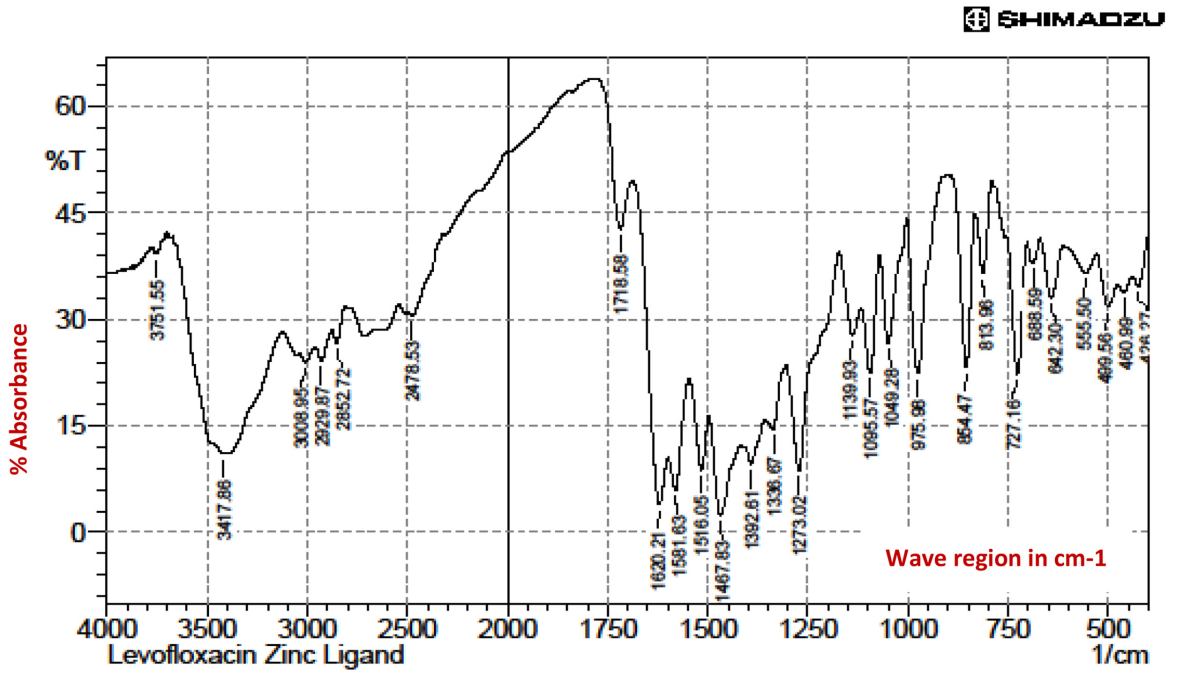
3.3. ^{1}H-NMR

3.3.1. The ^{1}H-NMR spectrum of both pure levofloxacin and zinc-levofloxacin ligand

This method can be applied to research, quality control, or the identification of unknowns. It can also be used to ascertain the purity and composition of a material (Sultana *et al.*, 2013). When compared to the signals of free levofloxacin, the ^{1}HNMR spectra of the ligand showed broader signals. The majority of the hydrogen atoms in the levofloxacin molecule exhibit an obvious shift to the downfield region in the zinc complex spectrum, where (H13) was shifted from (8.96 ppm) to (8.91 ppm), (H9) shifted from (7.58 ppm) to (7.63 ppm), (H3) was shifted also from (4.40 ppm) to (4.39 ppm), (H2) shifted from (4.94 ppm) to (4.89 ppm), (CH–CH3) was not shifted from (1.45 ppm) (Table 1). The shape and strength of the signal for each proton clearly changed along with this shift.

Table 1. H-MNR chemical shifts (ppm) from spectra of levofloxacin, zinc-levofloxacin ligand.

^{1}HNMR	levofloxacin	1,10-phenanthroline	Zinc-levofloxacin ligand
H13	8.96		8.91
H9	7.58		7.63
CH3 14	1.45		1.45
CH3′ 25	2.24		2.27
H2	4.94		4.89
H3	4.40		4.39
H 19, 17	2.45		2.44
H 20, 16	4.61	7.96	4.58
H3	–	7.77/7.74/7.73	7.81
H6	–	9.10/9.09	8.31/8.24/8.13
H1	–	8.48	9.16/8.94
H5	–		8.91



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	426.27	34.701	3.586	441.7	399.26	18.304	0.972
2	460.99	33.883	1.559	478.35	441.7	16.865	0.365
3	499.56	31.756	5.061	528.5	478.35	23.27	1.664
4	555.5	36.656	2.952	613.36	528.5	35.24	1.288
5	642.3	33.133	7.819	671.23	613.36	25.041	2.604
6	688.59	38.048	3.171	704.02	671.23	13.282	0.667
7	727.16	22.361	18.957	756.1	704.02	26.127	6.162
8	813.96	36.487	10.717	833.25	792.74	15.466	2.281
9	854.47	23.222	23.75	891.11	833.25	26.052	7.406
10	975.98	22.406	23.602	1002.98	902.69	43.372	10.793
11	1049.28	26.719	14.117	1072.42	1002.98	31.95	5.523
12	1095.57	22.499	12.802	1118.71	1072.42	25.427	4.377
13	1139.93	27.863	6.827	1172.72	1118.71	26.808	2.401
14	1273.02	8.785	18.67	1305.81	1172.72	87.183	18.537
15	1336.67	14.438	4.459	1357.89	1305.81	39.235	1.944
16	1392.61	9.595	3.494	1406.11	1357.89	44.214	2.703
17	1467.83	2.344	12.586	1496.76	1419.61	88.1	22.788
18	1516.05	8.824	9.683	1546.91	1496.76	42.893	6.639
19	1581.63	5.919	8.31	1598.99	1546.91	49.579	6.807
20	1620.21	3.928	16.106	1685.79	1598.99	67.787	12.06
21	1718.58	42.807	12.16	1772.58	1685.79	25.159	3.487
22	2478.53	30.609	1.663	2499.75	2322.29	80.51	1.985
23	2852.72	26.596	3.137	2877.79	2812.21	35.575	1.36
24	2929.87	24.235	2.788	2964.59	2877.79	50.784	1.755
25	3008.95	23.986	1.616	3037.89	2964.59	44.295	1.021
26	3417.86	11.053	3.776	3469.94	3124.68	269.657	18.572
27	3751.55	39.233	0.624	3757.33	3720.69	14.502	0.089

Fig. 3. FT-IR of zinc-levofloxacin ligand (500-4000 cm^{-1}).

3.3.2. *Physiochemical properties*

The melting point, color, form, and solubility of both levofloxacin and zinc-levofloxacin ligand are indicated in Table 2.

3.3.3. *Cytotoxicity of the zinc-levofloxacin ligand and IC50 estimation*

The cytotoxicity of ligand against the HRT-18 cancerous cell line was determined using a

Table 2. physicochemical properties of levofloxacin and zinc-levofloxacin ligand.

Sample	Color	Melting point	Form	Solubility
Levofloxacin	Pale yellow	225–228C	Powder	Methanol, water
zinc-levofloxacin ligand	Pale yellow	260–263C	powder	Hot Methanol, DMSO, Diethyl ether, chloroform, water

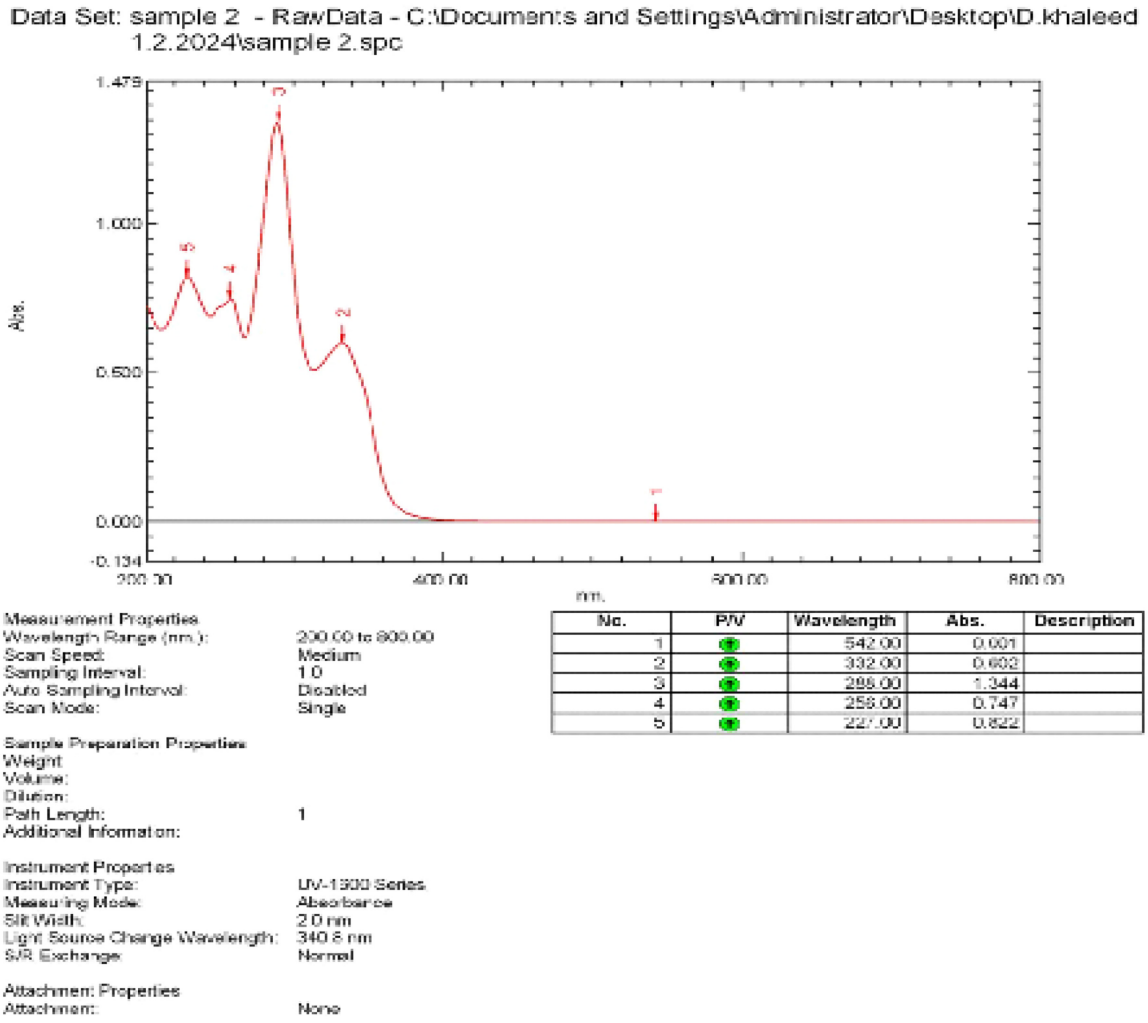


Fig. 4. UV-visible absorption spectra of pure Levofloxacin.

Methylthiotetrazolium (MTT) assay for assessment of the cytotoxicity of drugs which is also known as a cell viability assay. The cells were treated with various concentrations of zinc-levofloxacin ligand for 72 hours in a medium containing the complexes at concentrations of 31.25, 62.5, 125, 250, 500, and 1000 µg/mL.

The outcome demonstrated a significant 81.34 percent cytotoxic impact of zinc-levofloxacin ligand at 1000 µg/mL. Based on the coordination, the consequence is a considerable decrease in viable cells, as seen in Fig. 6.

The IC50 of zinc-levofloxacin ligand against HRT18 cancer cell line was calculated during a 72-hour ligand treatment period at different zinc-levofloxacin ligand concentrations; the cytotoxic concertation ranged from (62.5 to 1000) µg/ml (Fig. 6) and the IC50 was 79.42 µg/ml (Fig. 7).

The results of this study show that zinc-levofloxacin ligand has a high activity against the HRT18-cancerous cell line, which led to an effective IC50 value of 79.42 µg/ml.

4. Discussion

4.1. synthesis

In the synthesis of zinc-levofloxacin ligand, the two ionizable functional groups found in fluoroquinolones are the piperazine and carboxylic acid groups. Levofloxacin's nomenclature changes when a new ring is added between positions 1 and 8, changing the substituent locations to correspond with the new proposed nomenclature (Saour & Atto, 2012). Using the antibiotic levofloxacin and the (2N

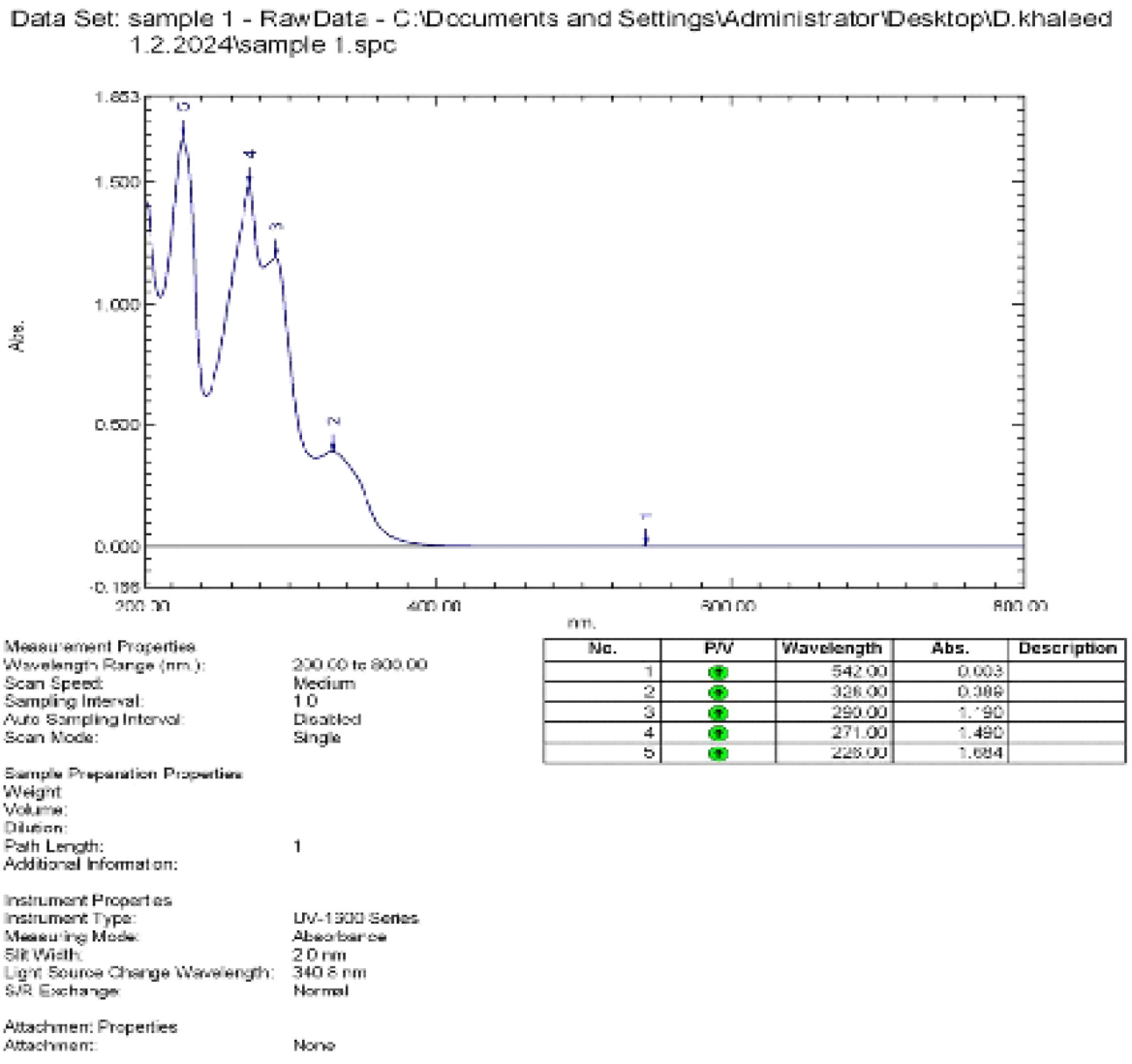


Fig. 5. UV-visible absorption spectra of zinc-levofloxacin ligand.

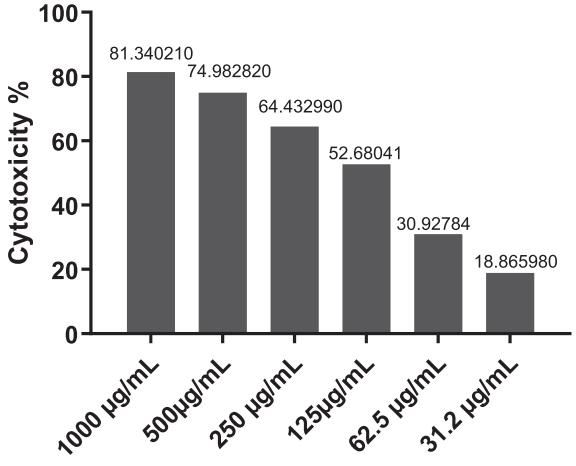


Fig. 6. Cytotoxicity effect of zinc-levofloxacin ligand in HRT cells.

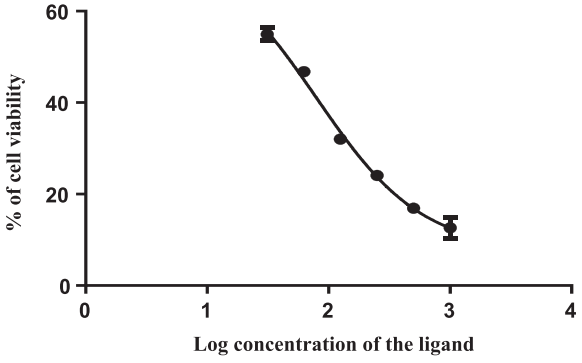


Fig. 7. Log concentration of zinc-levofloxacin ligand versus percentage of cell viability%. IC 50 of ligand on HRT-18 cell line, IC50 = 79.42 µg/mL. Cytotoxic concentration range = 125–1000 µg/mL

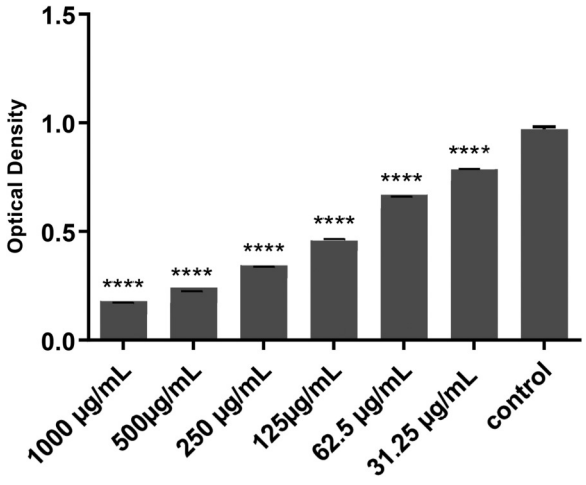


Fig. 8. Optical density of zinc-levofloxacin ligand on HRT-18 cancer cell line.

heterocyclic) chemical 1,10-phenanthroline, we were able to successfully construct a novel zinc complex.

Levofloxacin functions as a bivalent chemical, coordinating zinc through carbonyl and carboxyl oxygen molecules (Uivarosi, 2013). Meanwhile, phenanthroline coordinates the creation of the equatorial level by acting as a coordinator for two nitrogen atoms. To put it another way, zinc is essential for the binding and coordination of the ligand used in the synthesis of the new complex by two phenanthroline nitrogen atoms on one side and two ketone and carboxylic acid group oxygen atoms on the other side after losing their hydroxyl group from the levofloxacin drug's structure. This process was carried out using the Schiff base method (Tarab, 2022; Djurdjevic *et al.*, 2014).

4.2. Structural Characterization of zinc-levofloxacin ligand (FT-IR, ^{1}H-NMR, UV-visible analysis)

Proton Nuclear Magnetic Resonance (^{1}H-NMR), Fourier-transform infrared spectroscopy (FT-IR), and spectroscopic analysis were used to determine the complicated chemical structures.

Levofloxacin's infrared spectra are quite complex due to the presence of numerous functional groups in the molecules; therefore, their interpretation is based on the most common vibrations. The most significant region of the levofloxacin FTIR spectrum is found between 1800 and 1100 cm^{-1} (Tarab, 2022). Fourier transformation infrared spectra were used to analyze and determine the mechanism of coordination between levofloxacin, phenanthroline, and metal cations (Mubarak *et al.*, 2021). Levofloxacin showed a distinct characteristic. The carbonyl group's strong absorption band can be seen at 1722.43 cm^{-1}, while the pyridone stretch ν(c=o) may be found at 1620.09 cm^{-1} (Goyne *et al.*, 2005; Neugebauer *et al.*, 2005).

^{1}H-NMR was performed using NMR to collect both quantitative and qualitative information about the composition of a sample. This method can be applied to research, quality control, or the identification of unknowns. It can also be used to ascertain the purity and composition of a material (Sultana *et al.*, 2013). According to Drevenšek *et al.* (2006), the levofloxacin H-NMR spectra in DMSO shows signals at 8.96 ppm (H13), 7.58 ppm (H9), 8.48 ppm (H1), 9.10 ppm (H6), 4.40 ppm (H3), 4.94 ppm (H2), 2.45 ppm (H17, H19), 2.24 ppm (CH-CH3), and 1.45 ppm (CH-CH3). Because of the solvent exchange, the acidic proton of the carboxylic group (COOH) did not show up as a distinct signal (Ezugwu *et al.*, 2013).

The UV-Vis spectra of levofloxacin and zinc-levofloxacin ligand are similar except from slightly shifts and the samples have a high absorption capacity for electromagnetic radiation in the ultraviolet area. This fact is characteristic of the coordination of zinc-levofloxacin ligand with the metal ion through the oxygen atoms. More specifically, the zinc-levofloxacin ligand exhibits three intense bands in the

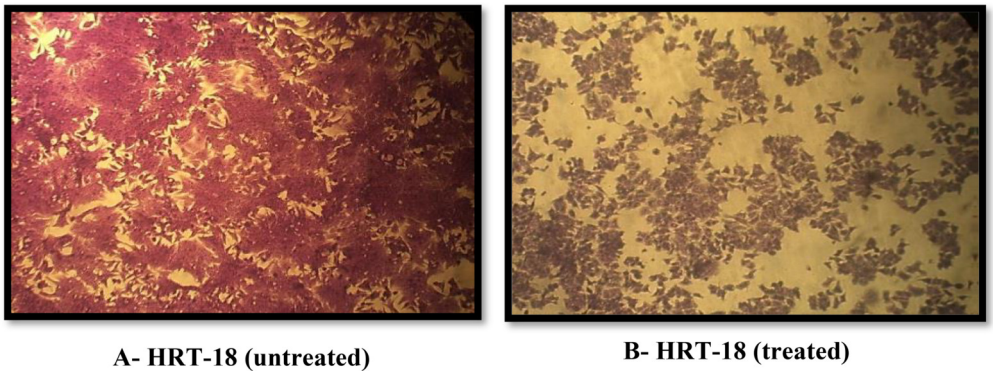


Fig. 9. Morphological picture for HRT-18 cancer cell line in vitro, (A) Control cells treated with DMSO (B) Cytotoxic effect of zinc-levofloxacin ligand under an inverted microscope, 10×.

ultraviolent region, due to transitions within the levofloxacin molecule. The fact that the UV-Vis spectra do not alter over time suggests that the compound in methanol is stable (Galani *et al.*, 2014). The broad peak is explained by the zinc particles in the ligand composition and phenomenon of the Surface Plasmon Resonance (SPR), which is caused by the free electrons in the tiny particles moving collectively when light strikes them (Jana *et al.*, 2016).

4.3. Cytotoxicity of the of zinc-levofloxacin ligand

Fluoroquinolone compounds were reported to cause programming cell death and decrease cell growth in experiments conducted on cancer cell lines (Mazandaran *et al.*, 2019). According to a number of studies (Mondal *et al.*, 2004; Cao *et al.*, 2017; Yadav & Talwar, 2019), fluoroquinolone may be useful in preventing the growth of lung adenocarcinoma and prostate cancer cell lines, as well as colorectal cancer cells (Melo *et al.*, 2011). According to studies conducted by El-Rayes *et al.* (2002) and Hallaq *et al.* (2022) some fluoroquinolones have been demonstrated to have anti-proliferative properties in *vitro* by stopping potential cancer cells from going through a biochemical transformation that increases the uptake of other chemotherapeutics and/or mediates immunomodulatory responses. While the other types that do not contain tetracyclic groups in their structures are not toxic to normal human dermal fibroblasts, several novel tetra-cyclic fluoroquinolones, like levofloxacin, have been found to have anticancer properties against a variety of human cells, including breast cancer cell line and non-small lung cancerous cell line (A549) (Al-Trawneh *et al.*, 2010).

The zinc-levofloxacin ligand has been shown in numerous studies to significantly decrease cell proliferation in the presence of fluoroquinolone antibiotics, which may explain why it can induce a high rate of apoptosis (Anderson & Osheroff, 2001; Herold *et al.*, 2002). Alternatively, it might be because it inhibits DNA synthesis by preventing supercoiling and strand segregation, which is mediated by topoisomerase II inhibition and enhances DNA cleavage in eukaryotic cells (Pommier *et al.*, 2010; Robinson *et al.*, 1992). Furthermore, fluoroquinolones, such levofloxacin, have demonstrated selective activity against certain cancer cells and are efficient inhibitors of tubulin polymerization (Chen *et al.*, 2007). The cell-killing effect of zinc-levofloxacin ligand on the HRT-18 cancerous cell line was measured using a Methylthiotetrazolium (MTT) assay, which is a type of cell viability assay used to assess the cytotoxicity of drugs.

The IC50 value, which represents the concentration of chemicals or medications that reduce cell viability or survival by 50%, is used to express cytotoxicity levels. The cytotoxic concentration of zinc-levofloxacin ligand was demonstrated to be many times stronger than that of levofloxacin which stated in other studies (Ahadi *et al.*, 2023), and its IC50 was found to be significantly lower than that of the drug itself. These findings indicate the ligand's effectiveness against HRT-118 colorectal cancer cells, and they may also have some effect on other cancer cell lines like human breast adenocarcinoma cells (MCF-7) (Galani *et al.*, 2014). However, more research is required to determine the precise mechanism of action of these ligands on other cancer cell lines (Huang *et al.*, 2018).

Levofloxacin inhibits DNA helicase function, preventing bacteria from copying their DNA. Due to the fact that mammalian cells share many intracellular biologic characteristic traits with prokaryotic cells, we assume that antibiotics that limit DNA duplication in prokaryotic cells may similarly impair the survivability of cancer cells. By causing a cell cycle arrest at G2-M and promoting apoptosis in the drug-exposed cells, levofloxacin significantly inhibits the growth of cancer cells, the creation of clones, and the development of tumors in xenografts (He *et al.*, 2022).

When the HRT-18 cancer cell line was exposed to various effective concentrations of zinc-levofloxacin ligand, the ligand effect on the cells was also seen under an inverted microscope. The zinc-levofloxacin ligand exhibited remarkable and targeted cytotoxic activity against the HRT-18 colorectal cancer cell line, leading to a significant decrease in cell viability in comparison to the control group. The HRT-18 colorectal cancer cell line was subjected to a strong lethal effect by zinc-levofloxacin ligand in this work. This effect may have been caused by pure levofloxacin, which binds to DNA helicase activity and inhibits it, reducing DNA duplication and also contributing to 1,10-Phenanthroline. The comprehensive structural-planer arrangement brought about by the zinc chelation with the 1,10-phenanthroline molecule may be the cause of zinc-levofloxacin ligand's extreme cytotoxicity. The notable intercalative chelation of zinc with DNA molecules may be the cause of this phenomenon. Thus, adding 1,10-Phenanthroline to zinc complexes may increase the activity and result in a more powerful anticancer medication. Additionally, metal chelates including fluoroquinolones play a significant role in topoisomerase toxicity and the augmentation of antineoplastic efficacy (Abdel-Aal *et al.*, 2019).

According to this research, zinc-levofloxacin ligand functions as a more potent anticancer, acting on the adenocarcinoma cell line after 72 hours of incubation. The higher concentrations (1000 µg/mL) significantly

inhibited the growth of the tested HRT-18 cell lines; this is likely because caspase (9) and (3) induce apoptosis, which is thought to be the cause of cell growth inhibition (Jantová *et al.*, 2018). Zinc-levofloxacin ligand powder that was created has the ability to infiltrate HRT-18 adenocarcinoma cells. When HRT-18 cells were treated with zinc-levofloxacin ligand powder, there was a notable reduction in their proliferation when compared to the control group that received DMSO. Consequently, these results might offer a novel therapeutic approach for the treatment of colon cancer.

5. Conclusion

This work examined the synthesis, characterization, and cytotoxic evaluation of zinc complexed with levofloxacin, a third-generation quinolone, and 1, 10-phenanthroline. Spectroscopic investigations of the zinc-levofloxacin ligand indicated above revealed that levofloxacin interacts with the zinc atom via one pyridone and one carboxylate oxygen from 1,10-phenanthroline.

The study concluded that the synthesized mixed complex (zinc-levofloxacin ligand) is a potential cytotoxic agent against HRT-18 colorectal cancer cell line.

Authors contributions

The study was conceptualized and designed by Prof Falah M K AL-Rekabi, who supervised the project, and contributed to the final version of the manuscript, Khalid Ibrahim Adwan performed the experiments and wrote the initial draft of the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgment

NA

Conflict of interest

The authors declare there is no conflict of interest.

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