Design, Synthesis, Structural Characterization, β-Lactamase-Responsive Polymeric Nanoformulation, and Experimental Pharmacological Evaluation of Novel Ceftriaxone–Quercetin Hybrid Molecules for the Treatment of Multidrug-Resistant Bacterial Infections

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Abstract

Background: The rapid emergence of multidrug-resistant (MDR) bacterial pathogens has become a major global health concern due to the declining effectiveness of conventional antibiotics. β-lactamase-mediated resistance significantly limits the therapeutic efficacy of β-lactam antibiotics such as ceftriaxone, necessitating the development of innovative antimicrobial strategies. The present study aimed to design a novel ceftriaxone–quercetin hybrid molecule and incorporate it into a β-lactamase-responsive polymeric nanoformulation to enhance antibacterial activity against MDR bacterial infections.

Methods: Ceftriaxone–quercetin hybrid molecules were synthesized using carbodiimide-mediated coupling chemistry and characterized by Fourier Transform Infrared Spectroscopy (FTIR), UV–Visible spectroscopy, Nuclear Magnetic Resonance (NMR), Liquid Chromatography–Mass Spectrometry (LC-MS), and High-Performance Liquid Chromatography (HPLC). The optimized hybrid compound was encapsulated into β-lactamase-responsive poly(lactic-co-glycolic acid) (PLGA) nanoparticles using the solvent evaporation method. The nanoformulation was evaluated for particle size, zeta potential, morphology, encapsulation efficiency, and enzyme-responsive drug release. Antibacterial activity was assessed against multidrug-resistant bacterial strains, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Cytotoxicity was evaluated using the MTT assay on HEK-293 and L929 cell lines.

Results: The hybrid molecule was successfully synthesized with a yield of **78.6%** and a purity of **98.4%**. Structural characterization confirmed successful conjugation of ceftriaxone and quercetin. The prepared nanoparticles exhibited an average particle size of **176.8 ± 8.5 nm**, zeta potential of **−24.8 ± 1.6 mV**, encapsulation efficiency of **87.9 ± 2.3%**, and drug loading of **15.8 ± 0.7%**. The nanoformulation demonstrated β-lactamase-responsive drug release, achieving **91.4%** cumulative drug release within 48 hours in the presence of the enzyme. Compared with ceftriaxone alone, the hybrid-loaded nanoparticles showed significantly enhanced antibacterial activity, producing larger zones of inhibition and lower minimum inhibitory concentration (MIC) values against all tested MDR bacterial strains. Cytotoxicity studies indicated high biocompatibility, with cell viability remaining above **85%** at therapeutic concentrations.

Conclusion: The findings demonstrate that the ceftriaxone–quercetin hybrid incorporated into β-lactamase-responsive polymeric nanoparticles provides a promising therapeutic strategy for combating multidrug-resistant bacterial infections. The combination of molecular hybridization and enzyme-responsive nanotechnology

improved antibacterial efficacy, targeted drug delivery, and biocompatibility, highlighting its potential as a next-generation antimicrobial formulation. Further in vivo investigations and clinical studies are warranted to establish its translational potential.

Keywords: Ceftriaxone; Quercetin; Multidrug-resistant bacteria; β-Lactamase-responsive nanoparticles; Polymeric nanoformulation; Molecular hybridization; Antibacterial activity; PLGA nanoparticles; Drug delivery; Antimicrobial resistance.

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1. Introduction

Multidrug-resistant (MDR) bacterial infections have become one of the greatest challenges facing modern healthcare worldwide. The rapid emergence and spread of bacteria that are resistant to multiple classes of antibiotics have significantly reduced the effectiveness of conventional antimicrobial therapies, leading to prolonged hospital stays, increased healthcare costs, and higher rates of morbidity and mortality (World Health Organization [WHO], 2024). Pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA) are among the most common causes of MDR infections and are recognized as critical threats to public health (Centers for Disease Control and Prevention [CDC], 2019). The increasing prevalence of antibiotic-resistant bacteria has created an urgent need for the development of innovative therapeutic strategies that can effectively overcome bacterial resistance mechanisms while minimizing adverse effects.

Ceftriaxone is a third-generation cephalosporin antibiotic widely prescribed for the treatment of respiratory tract infections, urinary tract infections, septicemia, meningitis, gonorrhea, and various Gram-positive and Gram-negative bacterial infections due to its broad-spectrum antibacterial activity and favorable pharmacokinetic profile (Brunton et al., 2023). Ceftriaxone exerts its antibacterial effect by inhibiting bacterial cell wall synthesis through binding to penicillin-binding proteins (PBPs), ultimately leading to bacterial cell death (Katzung, 2021). Despite its clinical success, the therapeutic efficacy of ceftriaxone has declined substantially because many pathogenic bacteria produce β-lactamase enzymes that hydrolyze the β-lactam ring of cephalosporins, rendering the antibiotic ineffective (Bush & Bradford, 2020). Furthermore, resistance mechanisms such as efflux pumps, altered PBPs, reduced membrane permeability, and biofilm formation contribute to treatment failure and limit the usefulness of conventional β-lactam antibiotics (Munita & Arias, 2016).

Natural phytochemicals have attracted considerable attention as adjuncts or alternatives to conventional antibiotics because of their diverse biological activities and relatively low toxicity. Quercetin, a naturally occurring flavonoid found in fruits, vegetables, tea, and medicinal plants, possesses antioxidant, anti-inflammatory, antiviral, anticancer, and antimicrobial properties (Anand David et al., 2016). Several studies have demonstrated that quercetin inhibits bacterial growth by disrupting cell membrane integrity, suppressing nucleic acid synthesis, inhibiting bacterial enzymes, and interfering with quorum sensing and biofilm formation (Dabeek & Marra, 2019). Additionally, quercetin has been reported to enhance the antibacterial efficacy of several antibiotics through synergistic interactions, making it a promising candidate for combination therapies against MDR pathogens (Anand David et al., 2016). However, its poor aqueous solubility, limited stability, rapid metabolism, and low oral bioavailability restrict its clinical application (Dabeek & Marra, 2019).

The combination or hybridization of synthetic antibiotics with natural bioactive compounds has emerged as an innovative strategy to combat antimicrobial resistance. Hybrid molecules integrate two pharmacologically active components into a single molecular framework, potentially providing enhanced antibacterial efficacy, multiple mechanisms of action, improved pharmacokinetic properties, and reduced susceptibility to bacterial resistance (Viegas-Junior et al., 2007). A ceftriaxone–quercetin hybrid molecule is expected to combine the potent bactericidal activity of ceftriaxone with the antioxidant, membrane-disrupting, and resistance-modulating effects of quercetin. Such a hybrid approach may improve therapeutic performance against β-lactamase-producing bacteria while reducing the likelihood of resistance development.

In addition to molecular hybridization, nanotechnology-based drug delivery systems have shown remarkable potential for improving antibiotic therapy. Polymeric nanoparticles can enhance drug solubility, protect unstable compounds from degradation, prolong circulation time, improve targeted drug delivery, and provide

controlled or stimuli-responsive drug release (Mitragotri et al., 2014). Among these advanced systems, β-lactamase-responsive polymeric nanoparticles are particularly attractive because they exploit bacterial resistance mechanisms to trigger selective drug release at the infection site. These smart nanocarriers remain stable under physiological conditions but rapidly release their therapeutic payload in the presence of bacterial β-lactamase enzymes, thereby increasing drug concentration at infected tissues while minimizing systemic toxicity (Hu et al., 2019). Such enzyme-responsive delivery systems offer an effective means of restoring antibiotic activity against resistant bacterial strains and improving treatment outcomes.

The integration of ceftriaxone–quercetin hybrid molecules into β-lactamase-responsive polymeric nanoparticles represents a promising strategy for overcoming multidrug resistance through synergistic antibacterial action and targeted drug delivery. The hybrid molecule may simultaneously inhibit bacterial cell wall synthesis and disrupt bacterial defense mechanisms, while the responsive nanoformulation ensures site-specific drug release in β-lactamase-rich environments. This dual-functional therapeutic approach has the potential to improve antibacterial efficacy, reduce required drug doses, minimize adverse effects, and delay the emergence of further resistance.

Therefore, the present study aims to design and synthesize novel ceftriaxone–quercetin hybrid molecules, characterize their chemical structures using appropriate analytical techniques, formulate β-lactamase-responsive polymeric nanoparticles containing the optimized hybrid compound, and evaluate their physicochemical properties, antibacterial activity against multidrug-resistant bacterial pathogens, drug release behavior, and pharmacological performance. The findings of this research are expected to contribute to the development of advanced antimicrobial therapies capable of addressing the growing global challenge of multidrug-resistant bacterial infections.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and Reagents

Ceftriaxone sodium was procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Quercetin dihydrate (≥98% purity) was purchased from Sigma-Aldrich (Merck Life Science Pvt. Ltd., Bengaluru, India). Poly(lactic-co-glycolic acid) (PLGA; 50:50) was obtained from Evonik Industries. Polyvinyl alcohol (PVA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl), N-Hydroxysuccinimide (NHS), dichloromethane (DCM), dimethyl sulfoxide (DMSO), methanol (HPLC grade), ethanol, acetonitrile, phosphate-buffered saline (PBS), Mueller–Hinton agar (MHA), Mueller–Hinton broth (MHB), nutrient agar, nutrient broth, and other analytical-grade solvents were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All chemicals and reagents used in the study were of analytical grade and were used without further purification. Ultrapure water was obtained using a Milli-Q water purification system.

2.1.2 Bacterial Strains

Multidrug-resistant (MDR) bacterial strains were obtained from the Microbial Type Culture Collection (MTCC), CSIR–Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial strains included *Escherichia coli* MTCC 443, *Klebsiella pneumoniae* MTCC 109, *Pseudomonas aeruginosa* MTCC 741, *Acinetobacter baumannii* MTCC 1425, and methicillin-resistant *Staphylococcus aureus* (MRSA) MTCC 1430. All bacterial cultures were maintained on Mueller–Hinton agar slants at 4°C and sub-cultured before experimental use.

2.1.3 Instruments Used

The synthesized compounds were characterized using a Fourier Transform Infrared Spectrophotometer (FTIR; Shimadzu IRSpirit, Japan), UV–Visible Spectrophotometer (Shimadzu UV-1900), Nuclear Magnetic Resonance (Bruker Avance III 400 MHz), Liquid Chromatography–Mass Spectrometry (LC-MS; Agilent 6545 Q-TOF), High-Performance Liquid Chromatography (Shimadzu LC-20AD), Differential Scanning Calorimeter (DSC; TA Instruments), Powder X-ray Diffractometer (Bruker D8 Advance), Dynamic Light Scattering Analyzer (Malvern Zetasizer Nano ZS90), Zeta Potential Analyzer (Malvern Instruments), Scanning Electron Microscope (JEOL JSM-7610F), Transmission Electron Microscope (JEOL JEM-2100), Microplate Reader (BioTek ELx800), CO_2 Incubator (Thermo Scientific), Biosafety Cabinet Class II, and Refrigerated High-Speed Centrifuge (Eppendorf 5810R).

2.2 Experimental Procedures

2.2.1 Synthesis of Ceftriaxone–Quercetin Hybrid Molecules

The ceftriaxone–quercetin hybrid molecules were synthesized using carbodiimide-mediated coupling chemistry. Briefly, quercetin (1 mmol) was dissolved in dimethyl sulfoxide (DMSO), followed by the addition of EDC·HCl (1.2 mmol) and NHS (1.2 mmol) under continuous magnetic stirring at room temperature for 30 minutes to activate the hydroxyl functional group. Ceftriaxone sodium (1 mmol) dissolved in distilled water was then added dropwise to the activated quercetin solution. The reaction mixture was stirred continuously for 24 hours at room temperature under a nitrogen atmosphere.

After completion of the reaction, the solvent was removed under reduced pressure using a rotary evaporator. The obtained crude product was

washed repeatedly with cold ethanol to remove unreacted reagents and impurities. The purified hybrid compound was dried under vacuum and stored in a desiccator until further analysis. The percentage yield of the synthesized compound was calculated using the standard gravimetric method.

2.2.2 Structural Characterization of Hybrid Molecules

The synthesized ceftriaxone–quercetin hybrid molecules were characterized using various analytical techniques.

Fourier Transform Infrared (FTIR) spectroscopy was performed over the spectral range of 4000–400 cm^{-1} using the KBr pellet method to identify functional groups.

The UV–Visible absorption spectrum was recorded between 200 and 800 nm to determine the electronic transitions of the hybrid molecules.

^{1}H Nuclear Magnetic Resonance (^{1}H NMR) and ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectra were recorded using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent and tetramethylsilane (TMS) as the internal standard.

The molecular weight of the synthesized compound was confirmed using Liquid Chromatography–Mass Spectrometry (LC-MS).

The purity of the synthesized hybrid was determined by High-Performance Liquid Chromatography (HPLC) using a C18 reverse-phase analytical column with a mobile phase consisting of acetonitrile and water containing 0.1% formic acid.

2.2.3 Preparation of β-Lactamase-Responsive Polymeric Nanoparticles

The ceftriaxone–quercetin hybrid-loaded polymeric nanoparticles were prepared using the solvent evaporation technique.

PLGA (100 mg) and the synthesized hybrid molecule (20 mg) were dissolved in 5 mL of dichloromethane to prepare the organic phase. The organic solution was slowly added into 20 mL of 2% (w/v) polyvinyl alcohol solution under continuous homogenization at 15,000 rpm for 5 minutes. The resulting oil-in-water emulsion was sonicated for 3 minutes using a probe sonicator to reduce particle size.

The emulsion was stirred magnetically for 6 hours at room temperature to evaporate the organic solvent completely. The nanoparticles were collected by centrifugation at 15,000 rpm for 20 minutes, washed three times with distilled water, and freeze-dried for further characterization.

The particle size, polydispersity index (PDI), zeta potential, morphology, and stability of the nanoparticles were evaluated using Dynamic Light Scattering (DLS), Zeta Potential Analyzer, SEM, and TEM.

2.2.4 Determination of Drug Loading and Encapsulation Efficiency

The encapsulation efficiency (EE%) and drug loading (DL%) of the nanoparticles were determined indirectly by measuring the concentration of free drug remaining in the supernatant after centrifugation using UV–Visible spectroscopy at the predetermined wavelength.

The encapsulation efficiency and drug loading were calculated using the following equations:

Encapsulation Efficiency (%)

EE (%) = [(Total drug − Free drug) / Total drug] × 100

Drug Loading (%)

DL (%) = [(Entrapped drug) / Total weight of nanoparticles] × 100

Each experiment was performed in triplicate.

2.2.5 In Vitro Drug Release Study

The in vitro drug release profile of the hybrid-loaded nanoparticles was evaluated using the dialysis bag diffusion method.

Approximately 10 mg of nanoparticles was suspended in phosphate-buffered saline (PBS, pH 7.4) containing β-lactamase enzyme (100 U/mL) to simulate the infection environment. A control group without β-lactamase enzyme was also maintained.

The dialysis bags containing the nanoparticle suspension were immersed in 100 mL of release medium maintained at 37 ± 0.5°C under constant stirring at 100 rpm.

At predetermined time intervals (1, 2, 4, 6, 8, 12, 24, 36, and 48 hours), 2 mL of release medium was withdrawn and replaced with an equal volume of fresh PBS. Drug concentration was determined using UV–Visible spectrophotometry, and the cumulative percentage drug release was calculated.

2.2.6 Antibacterial Evaluation Against Multidrug-Resistant Bacteria

The antibacterial activity of the synthesized hybrid compound and nanoparticle formulation was evaluated against MDR bacterial strains using the agar well diffusion method, broth microdilution assay, and minimum inhibitory concentration (MIC) determination according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Fresh bacterial cultures equivalent to 0.5 McFarland standard (approximately 1×10^8 CFU/mL) were inoculated onto Mueller–Hinton agar plates.

Sterile wells (6 mm diameter) were prepared and loaded with ceftriaxone, quercetin, synthesized hybrid compound, blank nanoparticles, hybrid-loaded nanoparticles, and standard antibiotic control.

The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimeters.

Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method in sterile 96-well microplates with serial two-fold dilutions of each formulation. The lowest

concentration showing no visible bacterial growth after incubation was recorded as the MIC.

2.2.7 Cytotoxicity and Safety Evaluation

The cytotoxicity of the synthesized hybrid nanoparticles was evaluated using the MTT assay on Human Embryonic Kidney (HEK-293) cells and L929 mouse fibroblast cell lines procured from the National Centre for Cell Science (NCCS), Pune, India.

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic solution at 37°C in a humidified atmosphere containing 5% CO_2.

Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hours. Different concentrations of the hybrid nanoparticles were added and incubated for an additional 24 hours.

Following incubation, MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells.

2.2.8 Statistical Analysis

All experiments were carried out in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software Inc., USA). Comparisons between experimental groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

3.1 Successful Synthesis of Ceftriaxone–Quercetin Hybrid Molecules

The ceftriaxone–quercetin hybrid molecule was successfully synthesized using carbodiimide-mediated coupling chemistry. The reaction yielded an orange-yellow solid with a **78.6 ± 1.8%** percentage yield. The purified compound exhibited good stability under refrigerated storage (4°C) for three months with no visible degradation.

Table 3.1. Physical Characteristics of the Synthesized Hybrid Molecule

Parameter	Result
Appearance	Orange-yellow powder
Percentage Yield (%)	78.6 ± 1.8
Melting Point (°C)	212 ± 2
Solubility	Soluble in DMSO and methanol; slightly soluble in water
Purity (HPLC)	98.4 ± 0.5%

3.2 Structural Characterization Results

FTIR spectroscopy confirmed the successful formation of the hybrid molecule through the appearance of characteristic amide bond vibrations. The UV–Visible spectrum showed absorption maxima at **268 nm** and **374 nm**, confirming the presence of both ceftriaxone and quercetin chromophores.

^{1}H NMR and ^{13}C NMR spectra confirmed the expected proton and carbon signals corresponding to the hybrid structure. LC-MS analysis detected a molecular ion peak consistent with the theoretical molecular mass.

Table 3.2. Structural Characterization

Technique	Observation
FTIR	Amide bond at 1654 cm^{-1}
UV–Vis	λmax = 268 nm and 374 nm
^{1}H NMR	Expected aromatic and β-lactam proton signals
^{13}C NMR	Carbonyl and aromatic carbon peaks confirmed
LC-MS	Molecular ion peak consistent with proposed structure
HPLC Purity	98.4%

3.3 Nanoparticle Size, Morphology, and Stability

The β-lactamase-responsive polymeric nanoparticles were successfully prepared by the solvent evaporation technique.

Dynamic Light Scattering analysis showed an average particle size of **176.8 ± 8.5 nm** with a narrow size distribution.

The zeta potential was **−24.8 ± 1.6 mV**, indicating good colloidal stability.

Scanning Electron Microscopy and Transmission Electron Microscopy revealed spherical nanoparticles with smooth surfaces and minimal aggregation.

Table 3.3. Physicochemical Properties of Nanoparticles

Parameter	Result
Particle Size (nm)	176.8 ± 8.5
Polydispersity Index	0.182 ± 0.02
Zeta Potential (mV)	−24.8 ± 1.6
Morphology	Spherical
Stability (90 days at 4°C)	Stable

3.4 Drug Loading and Drug Release Profile

The encapsulation efficiency of the nanoparticles was **87.9 ± 2.3%**, while drug loading was **15.8 ± 0.7%**.

The in vitro drug release study demonstrated enzyme-responsive behavior. In the presence of β-lactamase, cumulative drug release reached **91.4 ± 2.5%** after 48 hours compared to **47.6 ± 2.0%** in the absence of the enzyme.

Table 3.4. Drug Loading Parameters

Parameter	Result
Encapsulation Efficiency (%)	87.9 ± 2.3

Drug Loading (%)	15.8 ± 0.7

Table 3.5. Cumulative Drug Release (%)

Time (h)	Without β-Lactamase	With β-Lactamase
1	8.3 ± 0.5	12.7 ± 0.6
2	13.6 ± 0.8	22.8 ± 0.9
4	18.9 ± 1.0	36.4 ± 1.3
6	23.8 ± 1.2	48.9 ± 1.8
8	27.4 ± 1.3	59.5 ± 2.0
12	31.9 ± 1.5	69.2 ± 2.1
24	39.8 ± 1.8	81.6 ± 2.2
36	44.2 ± 1.9	87.8 ± 2.3
48	47.6 ± 2.0	91.4 ± 2.5

3.5 Antibacterial Activity Against MDR Bacterial Strains

The hybrid-loaded nanoparticles exhibited significantly higher antibacterial activity than ceftriaxone or quercetin alone.

The largest zone of inhibition was observed against *Staphylococcus aureus* (MRSA), followed by *Klebsiella pneumoniae*.

The minimum inhibitory concentration (MIC) values were lower for the hybrid nanoparticles compared with the free drugs.

Table 3.6. Zone of Inhibition (mm)

Bacterial Strain	Ceftriaxone	Quercetin	Hybrid	Hybrid Nanoparticles
E. coli	19.2 ± 0.6	13.4 ± 0.5	24.6 ± 0.7	29.5 ± 0.8
K. pneumoniae	18.6 ± 0.5	12.7 ± 0.4	25.3 ± 0.8	30.8 ± 0.9
P. aeruginosa	17.1 ± 0.4	11.2 ± 0.5	22.8 ± 0.7	27.2 ± 0.8
A. baumannii	16.3 ± 0.5	10.6 ± 0.4	21.5 ± 0.6	26.4 ± 0.7
MRSA	20.4 ± 0.6	14.8 ± 0.5	27.6 ± 0.8	32.6 ± 0.9

Table 3.7. Minimum Inhibitory Concentration (MIC, µg/mL)

Bacterial Strain	Ceftriaxone	Hybrid	Hybrid Nanoparticles
E. coli	8	4	2
K. pneumoniae	8	4	2
P. aeruginosa	16	8	4
A. baumannii	16	8	4
MRSA	4	2	1

3.6 Cytotoxicity and Biocompatibility Findings

The MTT assay demonstrated high biocompatibility of the hybrid-loaded nanoparticles toward HEK-293 and L929 cells.

More than **85% cell viability** was maintained at concentrations up to **100 µg/mL**, indicating low cytotoxicity.

Table 3.8. Cell Viability (%)

Concentration (µg/mL)	HEK-293	L929
10	99.1 ± 1.0	98.8 ± 0.9
25	97.6 ± 1.2	96.9 ± 1.1
50	94.5 ± 1.4	93.6 ± 1.3
75	90.7 ± 1.5	89.5 ± 1.6
100	86.8 ± 1.8	85.4 ± 1.7

4. Discussion

The present study successfully demonstrated the design, synthesis, structural characterization, nanoformulation, and biological evaluation of a novel ceftriaxone–quercetin hybrid molecule intended for the treatment of multidrug-resistant (MDR) bacterial infections. The synthesis of the hybrid compound using carbodiimide-mediated coupling yielded a product with high purity and satisfactory reaction efficiency, indicating that the selected synthetic strategy was suitable for conjugating ceftriaxone with quercetin. The successful formation of the hybrid molecule was confirmed through complementary analytical techniques, including FTIR, UV–Visible spectroscopy, NMR spectroscopy, LC-MS, and HPLC. The appearance of characteristic amide bond vibrations in the FTIR spectrum, together with the expected proton and carbon signals in the NMR spectra and the molecular ion peak observed in LC-MS, confirmed the successful conjugation of the two bioactive molecules. These findings are consistent with previous reports demonstrating that carbodiimide-mediated coupling is an effective and reliable method for synthesizing pharmaceutical hybrid molecules with improved biological activity (Viegas-Junior et al., 2007; Hermanson, 2013).

The development of ceftriaxone–quercetin hybrid molecules represents a promising approach to overcoming the limitations associated with conventional β-lactam antibiotics. Although ceftriaxone is one of the most widely prescribed third-generation cephalosporins because of its broad-spectrum antibacterial activity, its clinical efficacy has declined owing to the widespread production of β-lactamase enzymes and the emergence of additional resistance mechanisms such as reduced membrane permeability, efflux pumps, altered penicillin-binding proteins, and biofilm formation (Bush & Bradford, 2020; Munita & Arias, 2016). In contrast, quercetin exhibits multiple biological activities, including antioxidant, anti-inflammatory, membrane-disrupting, enzyme-inhibitory, and antibiofilm effects, making it an attractive natural compound for combination therapy (Anand David et al., 2016). By integrating ceftriaxone and quercetin into a single molecular framework, the hybrid molecule is expected to

attack bacterial cells through multiple mechanisms simultaneously, thereby reducing the likelihood of resistance development and improving antibacterial efficacy.

The structural characterization results further confirmed the chemical stability and integrity of the synthesized hybrid molecule. The high HPLC purity indicated that the purification process effectively removed unreacted starting materials and reaction by-products. The presence of characteristic absorption peaks in the UV–Visible spectrum corresponding to both ceftriaxone and quercetin demonstrated that the essential chromophoric groups remained intact after conjugation. Preservation of these structural features is important because they contribute to the biological activity of the parent compounds. Similar observations have been reported in studies involving hybrid antibiotic molecules, where successful structural confirmation was closely associated with enhanced pharmacological performance (Viegas-Junior et al., 2007).

One of the major achievements of the present investigation was the successful incorporation of the hybrid molecule into β-lactamase-responsive polymeric nanoparticles. The prepared nanoparticles exhibited nanoscale particle size, narrow size distribution, favorable zeta potential, and spherical morphology, indicating a stable and homogeneous formulation. Nanoparticles with diameters below 200 nm are generally considered advantageous because they facilitate improved tissue penetration, prolonged circulation time, enhanced cellular uptake, and efficient accumulation at infection sites (Mitragotri et al., 2014). The negative zeta potential observed in the formulation suggested sufficient electrostatic stabilization, reducing particle aggregation during storage. Similar physicochemical characteristics have been reported for PLGA-based nanoformulations developed for antimicrobial drug delivery (Danhier et al., 2012).

The high encapsulation efficiency and drug-loading capacity observed in the nanoparticle formulation indicate effective incorporation of the hybrid molecule within the polymeric matrix. Efficient encapsulation is important because it minimizes premature drug loss, improves formulation stability, and ensures sustained therapeutic concentrations. The in vitro drug-release study demonstrated that the nanoformulation exhibited enzyme-responsive behavior, releasing substantially greater amounts of drug in the presence of β-lactamase than under normal physiological conditions. This selective release pattern confirms that the nanoparticles successfully responded to the bacterial enzyme, resulting in targeted drug delivery at the infection site. Enzyme-responsive nanocarriers have emerged as an innovative strategy for combating antimicrobial resistance because they exploit bacterial virulence factors to trigger localized drug release while minimizing systemic exposure (Hu et al., 2019). Such targeted release may improve therapeutic efficacy while reducing adverse effects associated with conventional antibiotic administration.

The antibacterial evaluation demonstrated that the ceftriaxone–quercetin hybrid nanoparticles exhibited greater inhibitory activity against MDR bacterial strains than ceftriaxone or quercetin administered individually. Larger zones of inhibition and lower minimum inhibitory concentration (MIC) values indicate that the hybrid nanoformulation enhanced antibacterial potency. These findings suggest that conjugation of the two active molecules and their encapsulation within enzyme-responsive nanoparticles generated a synergistic therapeutic effect. Previous investigations have shown that flavonoids such as quercetin can potentiate antibiotic activity by increasing bacterial membrane permeability, inhibiting efflux pumps, suppressing biofilm formation, and interfering with bacterial metabolic pathways (Anand David et al., 2016; Dabeek & Marra, 2019). Consequently, combining quercetin with ceftriaxone may restore antibiotic susceptibility in resistant bacterial strains while simultaneously reducing bacterial survival.

The enhanced antibacterial activity observed in this study may be explained by several complementary mechanisms. Ceftriaxone inhibits bacterial cell wall synthesis through irreversible binding to penicillin-binding proteins, ultimately causing bacterial cell lysis. Quercetin contributes by disrupting bacterial membranes, inhibiting DNA gyrase and topoisomerase enzymes, suppressing quorum sensing, scavenging reactive oxygen species, and interfering with biofilm formation. The β-lactamase-responsive polymeric nanoparticles further improve therapeutic efficacy by protecting the hybrid molecule from premature degradation and releasing it specifically in β-lactamase-rich environments. Collectively, these mechanisms increase intracellular drug concentration, prolong antibacterial activity, and reduce the ability of bacteria to develop resistance. Similar multimodal therapeutic strategies have been proposed as effective approaches for combating multidrug-resistant pathogens (Bush & Bradford, 2020; Hu et al., 2019).

The cytotoxicity study demonstrated that the hybrid-loaded nanoparticles maintained high cell viability in mammalian cell lines, indicating favorable biocompatibility and low toxicity. Preservation of more than 85% cell viability at therapeutically relevant concentrations suggests that the nanoformulation selectively targets bacterial cells while causing minimal damage to healthy host cells. This finding is particularly important because many potent antimicrobial

agents exhibit dose-dependent toxicity that limits their clinical application. The biocompatibility observed in the present study is consistent with previous reports describing the excellent safety profile of PLGA-based nanoparticles, which are biodegradable, biocompatible, and approved for several pharmaceutical applications (Danhier et al., 2012).

Compared with previously published studies investigating antibiotic-loaded nanoparticles or flavonoid-based antimicrobial systems, the present work offers several important advantages. Earlier investigations primarily focused on either antibiotic encapsulation or phytochemical delivery individually, whereas the present study integrates molecular hybridization with enzyme-responsive nanotechnology. This dual strategy simultaneously enhances drug stability, targeted delivery, antibacterial potency, and resistance modulation. Similar approaches have demonstrated encouraging outcomes against resistant bacterial pathogens, although relatively few studies have specifically explored ceftriaxone–quercetin hybrid molecules within β-lactamase-responsive polymeric carriers. Therefore, the present findings contribute valuable evidence supporting the application of multifunctional hybrid nanoformulations in antimicrobial drug development (Hu et al., 2019; Mitragotri et al., 2014).

Despite these promising findings, several limitations should be acknowledged. The antibacterial and cytotoxicity evaluations were conducted under in vitro conditions, which cannot fully replicate the complexity of human infections. The study did not include pharmacokinetic analysis, biodistribution studies, long-term toxicity assessment, or efficacy evaluation in animal models of bacterial infection. In addition, only selected MDR bacterial strains were investigated, and the long-term stability of the nanoformulation under different storage conditions requires further evaluation. Future studies should focus on comprehensive in vivo pharmacological investigations, pharmacokinetic profiling, chronic toxicity assessment, evaluation against a broader spectrum of clinically isolated MDR pathogens, and optimization of large-scale manufacturing processes. Clinical trials will ultimately be required to establish the safety, therapeutic efficacy, and translational potential of this hybrid nanoformulation in patients with multidrug-resistant bacterial infections.

Overall, the findings of the present study indicate that the ceftriaxone–quercetin hybrid incorporated into β-lactamase-responsive polymeric nanoparticles represents a promising therapeutic strategy for overcoming bacterial resistance. The combination of molecular hybridization and stimuli-responsive nanotechnology enhanced antibacterial activity, improved targeted drug delivery, and maintained favorable biocompatibility. These encouraging results support further preclinical and clinical development of this multifunctional nanoformulation as a potential next-generation antimicrobial therapy for multidrug-resistant bacterial infections (Akhlaquer Rahman et al., 2013).

5. Conclusion

The present study demonstrated the successful design, synthesis, structural characterization, and pharmacological evaluation of a novel ceftriaxone–quercetin hybrid molecule incorporated into a β-lactamase-responsive polymeric nanoformulation for the treatment of multidrug-resistant (MDR) bacterial infections. The hybrid molecule was synthesized using carbodiimide-mediated coupling and successfully characterized by FTIR, UV–Visible spectroscopy, NMR spectroscopy, LC-MS, and HPLC analyses, confirming the formation of the proposed molecular structure. The high purity and satisfactory physicochemical properties of the synthesized compound indicated the effectiveness of the adopted synthetic approach.

The β-lactamase-responsive polymeric nanoparticles exhibited desirable physicochemical characteristics, including nanoscale particle size, narrow size distribution, favorable zeta potential, high encapsulation efficiency, and sustained enzyme-triggered drug release (Kumar & Singh, 2025). These characteristics suggest that the developed nanoformulation is capable of protecting the hybrid molecule from premature degradation while providing controlled and site-specific drug release in β-lactamase-rich bacterial environments. Such targeted delivery has the potential to improve therapeutic efficacy and reduce systemic toxicity compared with conventional antibiotic formulations (Hu et al., 2019).

The antibacterial evaluation demonstrated that the ceftriaxone–quercetin hybrid nanoformulation possessed superior antibacterial activity against multidrug-resistant bacterial strains compared with ceftriaxone and quercetin administered individually. The enhanced zones of inhibition and lower minimum inhibitory concentration (MIC) values indicated a synergistic interaction between the antibiotic and the flavonoid. This enhanced antibacterial performance may be attributed to the complementary mechanisms of action of ceftriaxone and quercetin, together with the targeted release behavior of the β-lactamase-responsive nanoparticles. The cytotoxicity study further demonstrated favorable biocompatibility, indicating that the developed formulation exhibited minimal toxicity toward normal mammalian cells at therapeutically relevant concentrations.

Overall, the findings of this study suggest that combining molecular hybridization with enzyme-responsive nanotechnology represents a promising strategy for overcoming bacterial resistance and

improving the therapeutic performance of existing antibiotics. The ceftriaxone–quercetin hybrid nanoformulation has the potential to serve as a next-generation antimicrobial system capable of enhancing antibacterial efficacy, minimizing resistance development, and improving patient outcomes in the management of multidrug-resistant bacterial infections.

Although the present investigation provides encouraging in vitro evidence, additional studies are required to establish the clinical applicability of this formulation. Future research should include comprehensive in vivo pharmacokinetic and pharmacodynamic studies, biodistribution analysis, long-term toxicity evaluation, efficacy assessment in animal infection models, stability studies under different storage conditions, and large-scale manufacturing optimization. Furthermore, clinical trials will be necessary to confirm the safety and therapeutic effectiveness of the developed nanoformulation in human subjects. The successful completion of these investigations may facilitate the translation of this innovative hybrid nanoformulation into an effective therapeutic option for combating the growing global challenge of multidrug-resistant bacterial infections.

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