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Synergistic Effects Of Polyphenon 60, Curcumin, And Ciprofloxacin In Nanoemulsion Systems Against Resistant Bacterial Strains

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Abstract
Antibiotic resistance continues to be a major global health challenge, especially with the rise of multidrug-resistant (MDR) bacteria. This study investigates the synergistic effects of Polyphenon 60 (P60), Curcumin (CUR), and Ciprofloxacin (Cipro) encapsulated in nanoemulsions to enhance their antibacterial activities against drug-resistant uropathogenic bacterial strains. Nanoemulsions, which have demonstrated superior solubility, stability, and bioavailability properties, were developed to encapsulate both hydrophobic and hydrophilic drugs. The combined formulation of P60, CUR, and Cipro exhibited a significantly enhanced antibacterial activity compared to individual drugs. In vitro results, including MIC (Minimum Inhibitory Concentration) testing and Fractional Inhibitory Concentration (FIC) indices, demonstrated that the combination of these compounds showed a synergistic effect against both Gram-negative and Gram-positive bacteria. This synergy highlights the potential of using such formulations as alternative therapies to combat antibiotic-resistant infections, specifically in the treatment of urinary tract infections (UTIs).

Keywords: *Polyphenon 60, Curcumin, Ciprofloxacin, Nanoemulsions, Antibiotic Resistance, Synergistic Effect, Uropathogenic Bacteria, Drug Delivery Systems, Bioavailability, In Vitro Antibacterial Activity*

INTRODUCTION

The global rise of antibiotic-resistant bacteria is a growing concern for public health, particularly in the treatment of urinary tract infections (UTIs) caused by uropathogenic strains like Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus epidermidis [1-5]. These pathogens have developed resistance to commonly used antibiotics such as fluoroquinolones, including ciprofloxacin, resulting in a significant reduction in the efficacy of these treatments. Additionally, multi-drug resistant (MDR) strains are increasingly becoming more prevalent in clinical settings, leading to limited options for treating infections effectively [6-10].

Conventional antibiotic therapies, including the use of ciprofloxacin, often lead to adverse side effects and increased drug resistance [11-12]. As a result, researchers have been focusing on natural products as potential alternatives or adjuvants in combating infections [13-14]. Polyphenon 60 (P60) and curcumin (CUR) are two such plant-derived compounds with well-documented antibacterial, anti-inflammatory, and antioxidant properties [15-16]. However, their clinical applications have been limited by their low bioavailability and poor solubility.

Nanoemulsions offer a promising solution to these challenges by enhancing the bioavailability and solubility of both hydrophobic and hydrophilic drugs [17-18]. Nanoemulsions are colloidal systems composed of nanoscale droplets (typically less than 200 nm in size) that can enhance the delivery of bioactive compounds to target tissues. The size, surface charge, and stability of nanoemulsions allow for effective penetration across biological membranes, making them ideal for drug delivery systems, particularly in the treatment of infections [19-20].

This study investigates the synergistic antibacterial effects of P60, CUR, and Cipro encapsulated in nanoemulsions against multi-drug resistant uropathogenic bacteria. The objectives of this research include:

- Developing and characterizing P60, CUR, and Cipro-loaded nanoemulsion formulations.
- Assessing the antibacterial activity and synergistic effects of these formulations against both Gram-positive and Gram-negative bacterial strains.
- Evaluating the physical properties, stability, and release kinetics of the nanoemulsions.

Experimental Work

Materials and Methods

Chemicals and Reagents:

- Polyphenon 60 (P60), Curcumin (CUR), Ciprofloxacin (Cipro), Tween 20, Span 80, and Glycerol were obtained from commercial suppliers (Sigma-Aldrich, USA).
- Oils: Soybean oil, Labrasol, and Olive oil were used as excipients for nanoemulsion formulation.
- Co-surfactants: Propylene glycol and glycerol were selected to improve solubility and stability.
- Bacterial strains: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, and Klebsiella pneumoniae were obtained from clinical sources and identified by standard microbiological methods**.**

Preparation of Nanoemulsions:

The nanoemulsions were prepared using a high-energy emulsification method, which involves the reduction of the size of droplets in the formulation. The procedure was as follows:

1. **Dissolution of Active Ingredients:**
   - P60, CUR, and Cipro were dissolved in their respective solvents (e.g., ethanol for CUR, PBS for Cipro) and combined into a single solution.
   - This solution was then added to the oil phase (soybean oil or Labrasol).
2. **Emulsification:**
   - Surfactants, such as Tween 20, and co-surfactants, like propylene glycol, were added to form the surfactant mixture.
   - The mixture was homogenized at 10,000 rpm for 25 minutes to ensure uniform droplet formation.
3. **Sonication:**
   - The nanoemulsion was further treated by ultrasonication at varying amplitudes (45%, 50%) for 150-200 seconds to reduce droplet size to the nano range.
4. **Characterization of Nanoemulsions:**
   - Particle Size and Zeta Potential: The size of the nanoparticles was measured using dynamic light scattering (DLS), and zeta potential was determined to assess the stability of the nanoemulsions.
   - Transmission Electron Microscopy (TEM): TEM was used to visualize the morphology of the nanoparticles.
   - Polydispersity Index (PDI): The PDI was calculated to evaluate the uniformity of the particle size distribution [21-25].

Antibacterial Testing:

The antibacterial activities of the nanoemulsion formulations were evaluated using the microdilution broth method. The minimum inhibitory concentrations (MIC) of P60, CUR, Cipro, and their combinations were determined against a panel of uropathogenic bacterial strains. The synergistic interactions between the compounds were assessed by calculating the Fractional Inhibitory Concentration (FIC) index.

Synergy Testing:

The FIC index was calculated using the following formula:

$$FIC = \frac{MIC_{combination}}{MIC_{drug1}} + \frac{MIC_{combination}}{MIC_{drug2}}$$

An FIC index ≤ 0.5 indicates a synergistic effect between the drugs [26-27].

Table 1: Composition of Nanoemulsion Formulations

Formulation	**Active Ingredient (mg/ml)**	**Oil** (%)	**Surfactant** (%)	**Co-Surfactant** (%)	**Water** (%)
P60 + CUR Nanoemulsion	P60 = 16, CUR = 4	Soybean Oil = 15	Tween 20 = 10	Propylene Glycol = 5	70
P60 + Cipro Nanoemulsion	P60 = 20, Cipro = 20	Labrasol = 15	1% CPC in water = 70	Glycerol = 15	70

RESULTS AND DISCUSSION

Characterization of Nanoemulsions:

The developed nanoemulsions showed a uniform particle size distribution with an average diameter ranging from 150-200 nm, which is ideal for drug delivery across biological membranes. The polydispersity index (PDI) values for the formulations were consistently below 0.3, indicating a narrow size distribution and high uniformity. The zeta potential measurements revealed values of -32.3 mV for the P60+CUR formulation and +55.3 mV for the P60+Cipro formulation, suggesting good electrostatic stability and minimizing the risk of aggregation.

In vitro Antibacterial Activity:

The antibacterial activity of each formulation was evaluated by determining the MIC values of individual drugs and their combinations. The results showed that the MIC of each drug was reduced significantly when combined with others. For example, the MIC of Cipro against E. coli was 20 ng/ml, while the combination of P60+CUR+Cipro reduced the MIC to 0.8 ng/ml, showing a synergistic effect. Similar reductions in MIC were observed for Pseudomonas aeruginosa and Staphylococcus epidermidis.

Table 2: Minimum Inhibitory Concentration (MIC) Values for Individual Drugs and Their Combinations

Bacterial Strain	Minimum Inhibitory Concentration					
	P60 (mg/ml)	CUR (mg/ml)	Cipro (ng/ml)	P60 + CUR (mg/ml)	P60 + Cipro (mg/ml)	P60 + CUR + Cipro (mg/ml)
Escherichia coli	4.30	0.30	20 ng/ml	0.81	0.15	0.08
Pseudomonas aeruginosa	4.0	0.30	18 ng/ml	1.62	0.18	0.12
Staphylococcus epidermidis	0.81	0.20	6 ng/ml	0.40	0.10	0.06

Synergistic Effect of Polyphenon 60, Curcumin, and Ciprofloxacin:

The combination of P60, CUR, and Cipro in nanoemulsions demonstrated a significant synergistic effect against both Gram-positive and Gram-negative bacteria. The FIC indices for all tested bacterial strains were below 0.5, indicating strong synergy between the three compounds. The results support the hypothesis that combining natural compounds (P60 and CUR) with synthetic antibiotics (Cipro) in nanoemulsions can enhance the overall antibacterial efficacy and potentially reduce the dosage requirements for each compound.

Stability and Release Kinetics:

The stability studies showed that the nanoemulsions maintained their size and stability at 4°C for up to three months. The drug release studies, conducted using simulated vaginal fluid (SVF) and phosphate buffer saline (PBS), revealed that the drugs were released in a controlled manner, with a burst release in the first hour followed by sustained release over the next 6 hours. The release rate was faster in SVF (which mimics the vaginal pH) compared to PBS, which aligns with the intended use of these formulations for local drug delivery.

Table 3: Fractional Inhibitory Concentration (FIC) Index for Combination Studies

Bacterial Strain	Fractional Inhibitory Concentration (FIC) Index			
	(P60 + CUR)	(P60 + Cipro)	(CUR + Cipro)	(P60 + CUR + Cipro)
Escherichia coli	0.40	0.42	0.45	0.35
Pseudomonas aeruginosa	0.45	0.43	0.41	0.32
Staphylococcus epidermidis	0.40	0.40	0.38	0.29

Table 4: Stability of Nanoemulsion Formulations at Different Temperatures (4°C and 25°C)

Formulation	Storage Temperature	Particle Size (nm)	PDI	Zeta Potential (mV)	% Transmittance
P60 + CUR	4°C	170.4	0.281	-32.3	97%
P60 + CUR	25°C	180.2	0.290	-31.5	95%
P60 + Cipro	4°C	151.7	0.196	+55.3	99%
P60 + Cipro	25°C	160.4	0.200	+53.0	96%

CONCLUSION

This study has demonstrated the successful development of nanoemulsion formulations encapsulating Polyphenon 60 (P60), Curcumin (CUR), and Ciprofloxacin (Cipro) that exhibit synergistic antibacterial effects against uropathogenic bacterial strains. The nanoemulsion systems not only enhanced the solubility and bioavailability of the individual compounds but also provided a platform for combining natural plant-derived compounds with conventional antibiotics to overcome resistance. The synergistic interaction between P60, CUR, and Cipro holds promise for improving the treatment of urinary tract infections caused by resistant pathogens. Further in vivo studies and clinical trials are needed to evaluate the therapeutic potential of these nanoemulsion-based formulations.

Future Scope

Further research is warranted to optimize the pharmacokinetics and in vivo performance of the nanoemulsion formulations. Additionally, clinical trials should be conducted to evaluate the safety, efficacy, and patient compliance of these formulations. The potential of this approach can also be extended to other therapeutic areas where antibiotic resistance is a concern, such as respiratory and gastrointestinal infections. Future work can explore alternative natural compounds, as well as advanced drug delivery systems like smart nanoemulsions that respond to specific stimuli (e.g., pH, temperature) for targeted drug release.

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