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Enhancing the Antimicrobial Effect of Ciprofloxacin by Loading with Silica Nanoparticles Derived From Rice Straw: Spectral, Morphological, and Drug Release Studies

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Abstract

ANTIMICROBIAL resistance is a significant threat to both human and animal health, such as salmonellosis, brucellosis, and resistant *E. coli* and *S. aureus* infections. The growing resistance of these pathogens complicates treatment and raises healthcare costs, highlighting the urgent need for alternative therapeutic strategies. Therefore, this study aimed to prepare silica nanoparticles (SiO_2-NPs) from rice straw through acid leaching method, then ciprofloxacin, one of the most abundant broad-spectrum antibiotics used worldwide, was loaded to SiO_2-NPs. Comprehensive characterization of TEM, SEM, EDX, and FTIR confirmed successful SiO_2-NPs synthesis and effective drug incorporation. TEM analysis for unmodified SiO_2-NPs showed spherical particles with a size of 130–170 nm, while ciprofloxacin-loaded SiO_2-NPs (CIP@SiO_2-NPs) expanded to 340–640 nm, indicating successful encapsulation and surface modification. These nanoparticles' antibacterial efficacy was tested against Gram-positive and Gram-negative bacteria frequently involved with zoonotic illnesses. The finding revealed that the ciprofloxacin-loaded SiO_2-NPs exhibited superior antibacterial activity to free ciprofloxacin especially for Gram (+ve) bacterial strains. Furthermore, the study proves the efficacy of rice straw-derived SiO_2-NPs as an eco-friendly nanocarrier approach for improving antibiotic delivery that enhances the management of bacterial infections in veterinary medicine, minimizing the risk of zoonotic disease transmission to humans.

Keywords: Bacterial Infection, Zoonotic Infection, Ciprofloxacin, Silica Nanoparticles, Rice-Straw.

Introduction

Antimicrobial resistance (AMR) has causeda serious worldwide health concern, affecting the efficiency of antibiotics in both human and veterinary medicine [1, 2]. The misuse of antibiotics in clinical, agricultural, and animal care settings is a major contributor to the rising prevalence of AMR [3]. The extreme usage of antibiotics, generally in veterinary and aquaculture medicine, has had an essential influence on establishing and spreading resistant bacterial strains [4, 5]. Accordingly, AMR may cause the spread of many diseases transmitted to humans by consuming contaminated animal products or bacterial strains that affect humans by direct contact or environmental exposure, leading to severe health consequences [6, 7]. The essential need for novel therapeutic strategies to combat these resistant pathogens is more evident than ever [8, 9].

It was detected that Zoonotic diseases, transmitted from animals to humans, are a significant part of emerging infectious diseases. The World Health Organization (WHO) has recorded over 200 zoonotic diseases, many associated with antimicrobial-resistant pathogens [10]. The overuse of antibiotics in veterinary practices, especially for disease prevention and growth promotion in

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veterinary medicine, has been a key factor in the development of AMR [11, 12].

Furthermore, antibiotic-resistant bacteria can spread from animals to humans through various means, including direct contact, consumption of contaminated animal products, and environmental exposure [13]. For instance, urban wildlife like ducks and crows have been found to carry antibiotic-resistant bacteria, posing a risk to public health by contaminating the food chain [7]. To address the escalating AMR crisis, a One Health approach is crucial, emphasizing the interconnectedness of human, animal, and environmental health [14, 10].

Infections induced by resistant bacteria could lead to prolonged illness, increased veterinary costs, and, in severe cases, animal mortality [4]. Resistant bacteria in animals also act as reservoirs that can infect humans, complicating treatment [6]. As a result, implementing antimicrobial stewardship programs in veterinary settings is critical to encouraging ethical antibiotic use and preventing resistance development [5]. Alternative therapeutic strategies, with nanotechnology gaining attention for their potential in combating resistant pathogens [15, 16]. The unique physicochemical features of nanomaterial's (such as their high surface area and small size) offer multiple mechanisms to inhibit bacterial growth. [17]. Amorphous silica nanoparticles (SiO_2-NPs) have shown particular promise in antimicrobial applications due to their high surface area compared to other nanoparticles [19, 20]. SiO_2-NPs can encapsulate various therapeutic agents, including antibiotics, ensuring controlled release and targeted delivery to infection sites [19, 20]. These leading SiO_2-NPs enable them to interact with bacterial cell membranes, inducing disruption and bacterial cell death. SiO_2-NPs also generate reactive oxygen species (ROS), further enhancing their antimicrobial activity [16]. The antimicrobial properties and drug delivery capabilities of SiO_2-NPs offer a dual-functional approach to combating AMR [17].

The present study discusses loading ciprofloxacin onto the amorphous SiNPs surface to enhance its antimicrobial efficacy. Ciprofloxacin is a broad-spectrum bactericidal second-generation fluoroquinolone that inhibits crucial bacterial growth enzymes [21, 22]. Despite its efficacy, ciprofloxacin has potential side effects, and many efforts are now being made to develop resistant strains, emphasizing the need for responsible usage to maintain the effectiveness of ciprofloxacin [22]. This research aims to investigate aspects like drug release profiles, encapsulation efficacy, and the antimicrobial effect of ciprofloxacin-loaded SiO_2-NPs against resistant bacterial strains. The study findings seek to provide insights into enhancing the therapeutic efficacy of ciprofloxacin and offer solutions for combating AMR, particularly through advanced drug delivery systems like amorphous silica nanoparticles.

Material and Methods

Synthesis of Silica Nanoparticles

Silica nanoparticles (SiO_2-NPs) were synthesized via acid leaching of grinded rice straw, a method known for producing high-purity amorphous silica with substantial surface area [19]. Initially, 25 g of finely ground rice straw was immersed in 100 mL of concentrated hydrochloric acid (HCl) and stirred continuously for 3 hours at ambient temperature to remove metallic impurities. Post-leaching, the mixture was neutralized using sodium hydroxide (NaOH) until a neutral pH was achieved, followed by filtration to separate the solid residue. The obtained residue was oven-dried at 100 °C and subsequently subjected to calcination in a muffle furnace at 750 °C for 5 hours. This thermal treatment yielded a white, amorphous silica powder, which was stored at room temperature for further analysis and applications.

Surface Functionalization of Silica Nanoparticles

The synthesized SiO_2-NPs were functionalized using the crosslinker, (3-aminopropyl) trimethoxysilane (APTMS) to introduce amine groups onto their surfaces, enhancing their reactivity for subsequent drug loading. The functionalization process involved dispersing the SiO_2-NPs in an ethanol solution containing APTMS, followed by stirring for a specified duration to allow salinization. Post-reaction, the modified nanoparticles were washed thrice with distilled water and once with 70% ethanol to remove unreacted silane and by-products. The final product was dried in a petri dish, resulting in a white powder of amine-functionalized SiO_2-NPs.

Loading of Ciprofloxacin onto Functionalized Silica Nanoparticles

To load ciprofloxacin (CIP) onto the functionalized SiO_2-NPs, 0.16 g of CIP was dissolved in 100 mL of distilled water using magnetic stirring to ensure complete dissolution. Subsequently, 0.3 g of the amine-functionalized SiO_2-NPs was added to the solution, and the mixture was stirred at 80 °C for 3 hours to facilitate adsorption of CIP onto the nanoparticle surfaces. The CIP@SiO_2-NPs were then separated by centrifugation, washed twice with distilled water and once with 70% ethanol to remove unbound drug molecules, and dried in a petri dish. The supernatants from the washing steps were collected to determine drug loading efficiency.

Characterization of Silica Nanoparticles

The synthesized and functionalized SiO_2-NPs were comprehensively characterized using various analytical techniques. Transmission Electron Microscopy (TEM), EOL JEM-2010 (Japan) operated at 200 kV, was employed to investigate the

morphology and size distribution of three types of samples: unmodified SiO_2-NPs, amine-functionalized SiO_2-NPs, and CIP@SiO_2-NPs. The Energy-Dispersive X-ray Spectroscopy (EDX) was employed to analyze their elemental composition. Additionally, Fourier-Transform Infrared Spectroscopy (FTIR), JASCO spectrometer (Japan), was conducted to identify characteristic functional groups and to confirm the successful surface modification with amine groups as well as the subsequent loading of ciprofloxacin onto the nanoparticles. All characterizations were conducted at the institute of nanoscience and Nanotechnology, Kafr El-Sheikh University, Egypt.

Antibacterial Activity Assessment

The antibacterial efficacy of the CIP@SiO_2-NPs was assessed using the agar well-diffusion approach against four bacterial strains: *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), *Bacillus cereus* (Gram-positive), and *Escherichia coli* (Gram-negative). These strains were obtained from the Microbial Genetics Laboratory, Genetic Engineering and Biotechnology Division, National Research Centre, Egypt. Each bacterial strain was cultured in Mueller–Hinton broth and incubated at 35 °C with shaking at 120 rpm for 24 hours. The cultures were then adjusted to an optical density of 0.5 at 570 nm, corresponding to approximately 1.5×10^8 CFU/mL. Sterile cotton swabs spread the bacterial suspensions uniformly onto Mueller–Hinton agar plates. After that, wells (6 mm) were punched into the agar, and 100 µL of the CIP-loaded SiNP suspension was introduced into each well. The plates included tested samples were incubated for 24 hrs at 37 °C, after which the inhibition zones were measured to assess antibacterial activity.

Results

Characterization of Silica Nanoparticles

Transmission Electron Microscopy (TEM)

TEM images (Fig1. a,b) revealed that the synthesized silica nanoparticles (SiO_2-NPs) were predominantly spherical with uniform size distribution. The average particle size was estimated to be approximately 130 to 170, showing slight agglomeration nm. Post-functionalization with (3-aminopropyl)trimethoxysilane (APTMS), the TEM images (Fig1. c,d) showed SiO_2-NPs maintained their morphology, with a minor increase in apparent particle size due to surface coating, indicating successful surface modification without significant aggregation. On the other hand, the HR-TEM images (Fig1. e,f) for the CIP@SiO_2-NPs also retained their spherical shape, with a slight increase in size to approximately 340–640 nm, suggesting effective drug loading.

Energy-Dispersive X-ray Spectroscopy (EDX)

EDX pattern Fig.2. a, confirmed the elemental composition of the SiO_2-NPs , primarily consisting of silicon and oxygen, and minor Ca and P impurities may be due to the residual of rice straw. Fig.2. b, showed additional signals due to the presence of nitrogen in the functionalized SiO_2-NPs, indicating successful amine group incorporation, while the EDX pattern in Fig.2. c for the SiO_2-NPs - *APTMS loaded ciprofloxacin* detect fluorine atoms, confirmed drug loading.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra of unmodified SiO_2-NPs shows characteristic peaks at ~3400 cm^{-1} (O–H stretching), ~1630 cm^{-1} (H–O–H bending), ~1080 cm^{-1} (Si–O–Si asymmetric stretching), and ~800 cm^{-1} (Si–O–Si symmetric stretching), confirming the formation of amorphous silica. After surface modification with APTMS, new peaks at 2900–2950 cm^{-1} (C–H stretching) and ~1550 cm^{-1} (N–H bending) appear, indicating successful functionalization with amine groups. Following ciprofloxacin loading, additional bands at ~1700–1725 cm^{-1} (C=O stretching) and ~1450–1380 cm^{-1} (C–N and CH_3 bending) emerge, confirming the successful incorporation of ciprofloxacin onto the functionalized silica nanoparticles.

Drug Loading Efficiency

The loading efficiency of ciprofloxacin onto the functionalized SiO_2-NPs was determined by measuring the concentration of unbound drug in the supernatant post-loading. Using UV-Vis spectrophotometry at 276 nm, the loading efficiency was calculated to be 87%, indicating a substantial amount of drug was successfully adsorbed onto the nanoparticles.

Antibacterial Activity

The antimicrobial activity of the SiO_2-NPs - *APTMS loaded ciprofloxacin* (CIP) was evaluated using the agar well diffusion method against a panel of Gram-positive and Gram-negative bacterial strains. The results demonstrated that the CIP@SiO_2-NPs exhibited enhanced antibacterial activity compared to free ciprofloxacin against most tested strains. For Gram-positive bacteria, *Bacillus subtilis* and *Bacillus licheniformis* showed increased inhibition zones with CIP@SiO_2-NPs (16.29 mm and 19.21 mm, respectively) compared to ciprofloxacin alone (15.33 mm and 18.09 mm, respectively). Similarly, a slight improvement was observed against *Bacillus cereus* and *Staphylococcus aureus*. Among Gram-negative strains, a notable enhancement was detected for *Escherichia coli*, where the inhibition zone increased from 11.55 mm for free ciprofloxacin to 13.07 mm for the loaded nanoparticles. *Pseudomonas fluorescens* also exhibited a slight increase in the inhibition zone with CIP@SiO_2 NPs,

while *Pseudomonas aeruginosa* showed a minor decrease. No significant difference was observed for *Enterobacter ludwigii*. Inhibition zone diameters were expressed as mean values. To assess the effect of treatment, a Wilcoxon signed-rank **test** was performed comparing ciprofloxacin and SiO_2-NPs-APTMS loaded ciprofloxacin within each bacterial group. The analysis was conducted separately for Gram-positive (*Bacillus subtilis, B. licheniformis, B. cereus, Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, P. fluorescens, Enterobacter ludwigii*) strains. A statistically significant increase in inhibition zone diameter was observed in the Gram-positive group following nanoparticle encapsulation (p = 0.038), whereas the difference in Gram-negative strains was not significant (p = 0.875). These findings suggest that loading ciprofloxacin onto silica nanoparticles can improve its antibacterial efficacy compared to free ciprofloxacin, particularly against certain Gram-positive bacteria and specific Gram-negative strains such as *E. coli* and *P. fluorescens*.

Discussion

The results of this study provide important insights into the evolving application of nanoparticle-based drug delivery systems in veterinary antimicrobial therapy, particularly targeting zoonotic bacterial pathogens [14, 8]. The observed increase in particle size following ciprofloxacin encapsulation, as demonstrated by transmission electron microscopy (TEM), aligns with previous findings indicating that drug loading onto silica nanoparticles induces particle expansion. This size enlargement is associated with improved sustained drug release, a critical attribute for the effective management of chronic and recurrent infections in veterinary medicine [23, 24].The successful surface modification and encapsulation were further supported by elemental analysis measured by the EDX analysis, which detected elemental signatures critical for confirming functionalization [25]. In veterinary contexts, such modifications are particularly valuable for improving drug stability and enhancing bioavailability in complex biological environments such as infected tissues or abscesses in companion animals and livestock [26].

The spectral analysis including the Fourier-transform infrared spectroscopy (FTIR) data revealed the presence of Si–O–Si networks, amine groups, and ciprofloxacin functional bands, suggesting strong electrostatic interactions between the drug and the nanoparticle matrix. These findings align with previous studies highlighting that amine-functionalized SiO_2-NPs improve antibiotic loading and release kinetics, enhancing therapeutic outcomes in animal models [27].

The improved antimicrobial efficacy of CIP@SiO_2-NPs against Gram-positive and Gram-negative veterinary pathogens such as *Bacillus subtilis*, *Bacillus licheniformis*, and *Escherichia coli* demonstrates the potential of this platform in managing common animal infections. Therefore, by improved local drug concentration and sustained release the need for frequent dosing reduced, thereby minimizing stress in treated animals and decreasing the risk of antibiotic overuse, a major driver of antimicrobial resistance [28, 29].

The slightly reduced efficacy observed against *Pseudomonas aeruginosa*, a known multidrug-resistant organism in both human and veterinary medicine, underlines the importance of developing combination therapies or nanoparticle-based synergistic strategies to overcome intrinsic bacterial defense mechanisms [30, 31].

In addition to acting as carriers, SiO_2-NPs may exhibit intrinsic antimicrobial properties, such as membrane disruption and reactive oxygen species (ROS) generation [32, 16]. Such synergistic influences could be particularly beneficial in veterinary wound management, mastitis treatment in dairy cattle, and surgical site infection control, particularly in small animals.

Utilizing rice straw (biomass in Egypt) as a precursor for silica nanoparticles provides an environmentally sustainable and cost-effective approach. This strategy is highly relevant for veterinary applications in low and middle-income countries (such as Egypt), where there may be restricted access to therapeutics can be limited [33].

In summary, this study presents a promising, eco-friendly approach for enhancing the antimicrobial efficacy of conventional antibiotics through silica nanoparticle-based delivery systems. This strategy holds potential for addressing the urgent need for effective antimicrobial therapies in veterinary practice, particularly in the face of growing concerns about zoonotic disease transmission and antibiotic stewardship [14, 8].

Limitations for the present work: There are some challenges to apply this solution such as rigorous toxicity study should be done to assess the effect of this nano- drug delivery system on animal and human health. Also for future work, another preparation method parameters will be tested to get smaller SiO_2-NPs with enhanced antimicrobial activity.

Conclusion

In conclusion, SiO_2-NPs were successfully synthesized from rice straw through acid extraction processes, resulting high-purity amorphous SiO_2-NPs with desirable surface properties. Surface functionalization with (3-aminopropyl) trimethoxysilane (APTMS) was achieved, providing amine groups that facilitated the efficient loading of ciprofloxacin onto the nanoparticles. Comprehensive

characterization using TEM, SEM-EDX, and FTIR confirmed the successful synthesis, modification, and drug loading processes. Antibacterial assays demonstrated that ciprofloxacin-loaded SiO_2-NPs exhibited enhanced or comparable antimicrobial activity against a range bacterial strains, compared to free ciprofloxacin, particularly improving efficacy against *Escherichia coli*, *Bacillus subtilis*, and *Bacillus licheniformis*. These determinations emphasize the possibility of SiO_2-NPs being utilized as drug delivery systems for improving antibiotic performance and comprising new alternative antibiotics, including nano-sized materials.

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Declaration of Conflict of Interest

None of the authors have any conflicting interests

Authors' contributions

Rania Al wakeel , Ola El Borady the experimental work of the characterization part, investigation, data analysis, and writing of the original draft. Mohamed Abdelaal; supervision, validation, and reviewing of the final manuscript.

Ethical of approval

This study not need ethical regulations.

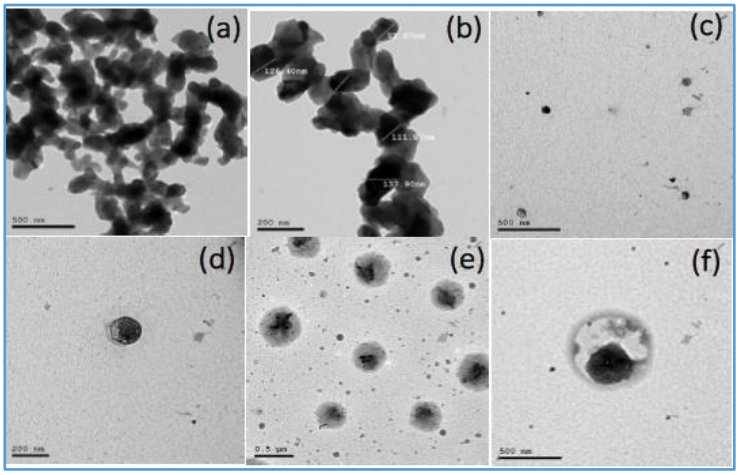

Fig. 1. (a,b) TEM images of unmodified SiO_2-NPs from different spots; (c, d). SiO_2-NPs after functionalization with (3-aminopropyl) trimethoxysilane (APTMS), (e,f) SiO_2-NPs - APTMS loaded ciprofloxacin.

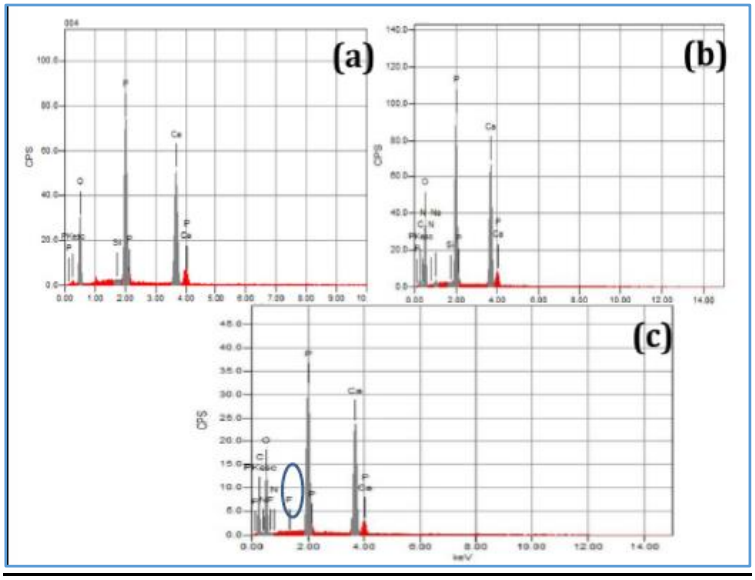

Fig. 2. (a) the EDX pattern of unmodified SiO_2-NPs, (b) EDX spectra APTMS-functionalized silica nanoparticles, (c) the EDX pattern of SiO_2-NPs - APTMS loaded ciprofloxacin.

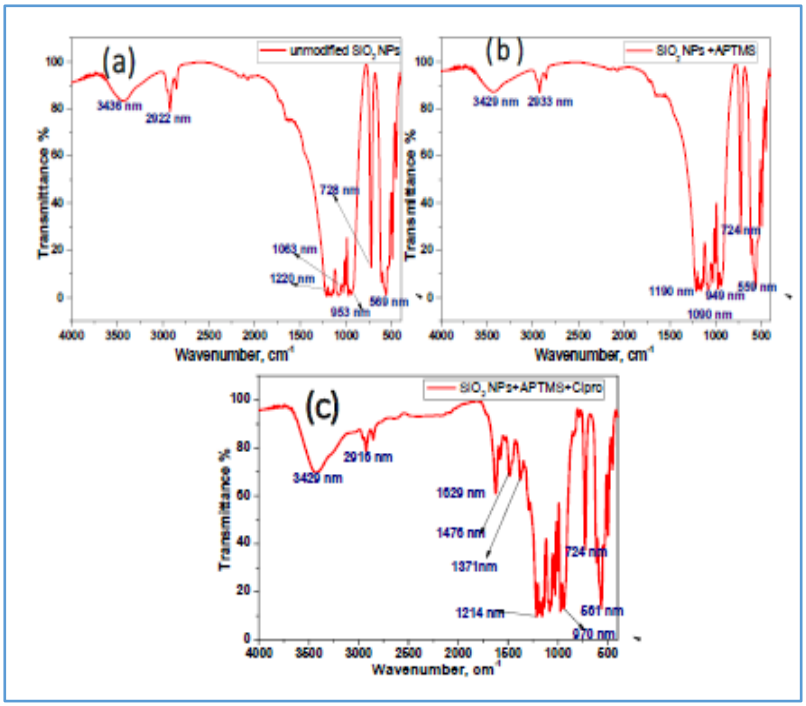

Fig. 3. FTIR spectra of (a) unmodified silica nanoparticles (SiO_2 NPs), (b functionalized silica nanoparticles using APTMS, and (c) ciprofloxacin-loaded silica nanoparticles

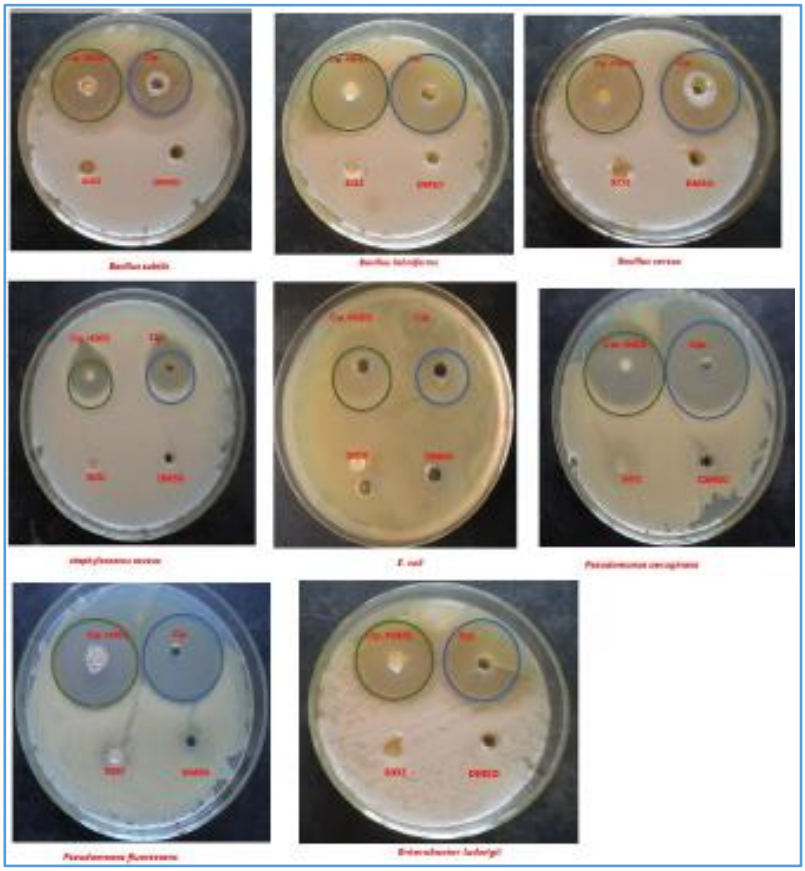

Fig. 4. Zone of inhibition diameters for ciprofloxacin alone and CIP@SiO_2 NPs against various Gram-positive and Gram-negative bacterial strains

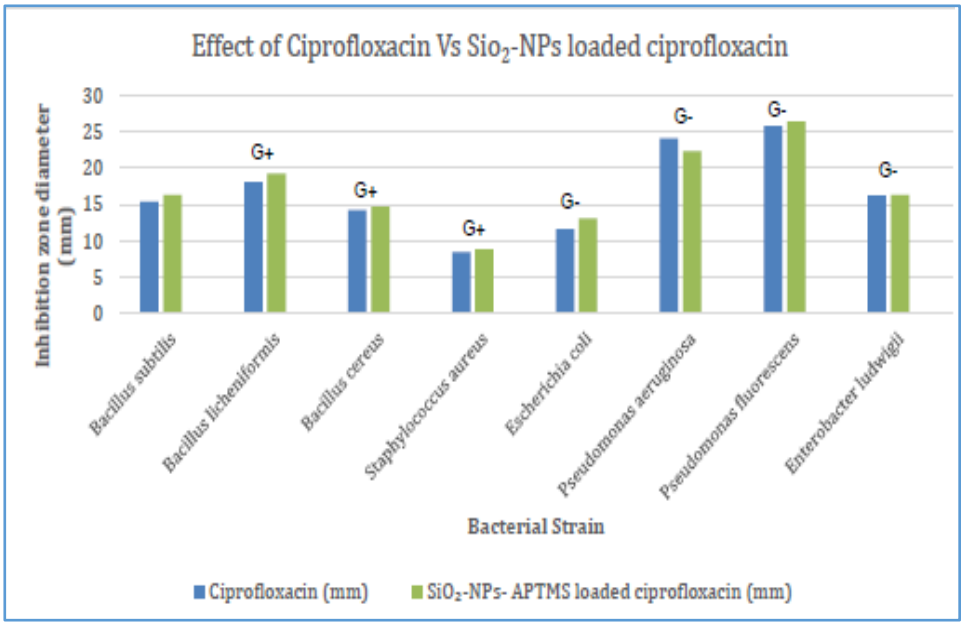

Fig. 5. Bar chart illustrating the inhibition zone diameters (in mm) of ciprofloxacin (blue bars) and SiO_2-NPs-APTMS loaded ciprofloxacin (green bars) against eight bacterial strains, including both Gram-positive (Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Staphylococcus aureus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens, Enterobacter ludwigii) organisms

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تعزيز التأثير المضاد للميكروبات للسيبروفلوكساسين عن طريق التحميل بجسيمات السيليكا النانوية المشتقة من قش الأرز: دراسات طيفية ومورفولوجية ودراسات إطلاق الدواء

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الملخص

تُشكل مقاومة مضادات الميكروبات تهديدًا كبيرًا لصحة الإنسان والحيوان، مثل داء السالمونيلا، وداء البروسيلا، وعدوى الإشريكية القولونية والمكورات العنقودية الذهبية المقاومة. تُعقّد المقاومة المتزايدة لهذه المُمْرِضات العلاج وترفع تكاليف الرعاية الصحية، مما يُبرز الحاجة المُلِحّة لاستراتيجيات علاجية بديلة. لذلك، هدفت هذه الدراسة إلى تحضير جسيمات نانوية من السيليكا (SiO_2-NPs) من قش الأرز باستخدام طريقة الاستخلاص الحمضي، ثم تم تحميل السيبروفلوكساسين، أحد أكثر المضادات الحيوية واسعة الطيف انتشارًا واستخدامًا عالميًا، في جسيمات نانوية من السيليكا .(SiO_2-NPs) وقد أكّد التوصيف الشامل لتقنيات المجهر الإلكتروني النافذ(TEM) ، والمجهر الإلكتروني الماسح(SEM) ، وEDX، و FTIRنجاح تخليق جسيمات النانو من السيليكا (SiO_2-NPs) وفعالية دمج الدواء. أظهر تحليل المجهر الإلكتروني النافذ لجسيمات النانو من السيليكا (SiO_2-NPs) غير المُعدّلة جسيمات كروية يتراوح حجمها بين 130 و170 نانومتر، بينما توسّعت جسيمات النانو من السيليكا (SiO_2-NPs) المُحمّلة بالسيبروفلوكساسين (CIP@SiO_2-NPs) إلى 340-640 نانومتر، مما يُشير إلى نجاح التغليف وتعديل السطح. تم اختبار فعالية هذه الجسيمات النانوية المضادة للبكتيريا ضد البكتيريا موجبة وسالبة الجرام التي غالبًا ما ترتبط بأمراض حيوانية المنشأ. وكشفت النتائج أن جسيمات أكسيد السيليكون النانوية المحملة بالسيبروفلوكساسين أظهرت نشاطًا مضادًا للبكتيريا يفوق السيبروفلوكساسين الحر، وخاصةً ضد سلالات البكتيريا الموجبة الجرام. علاوة على ذلك، أثبتت الدراسة فعالية جسيمات أكسيد السيليكون النانوية المشتقة من قش الأرز كنهج ناقل نانوي صديق للبيئة لتحسين توصيل المضادات الحيوية، مما يعزز إدارة العدوى البكتيرية في الطب البيطري، ويقلل من خطر انتقال الأمراض حيوانية المنشأ إلى البشر.

الكلمات الدالة: عدوى بكتيرية، عدوى حيوانية المنشأ، سيبروفلوكساسين، جسيمات السيليكا النانوية، قش الأرز