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Biopolymer zein-coated gold nanoparticles: Synthesis, antibacterial potential, toxicity and histopathological effects against the Zika virus vector *Aedes aegypti*

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ABSTRACT

The control of multidrug-resistant bacteria as well as insect pests and vectors is timely and important now a days. The present study was designed to evaluate the in vitro antibacterial, antibiofilm and mosquito larvicidal effects of gold nanoparticles synthesized using the zein biopolymer (Ze-AuNPs) against Gram positive (*Bacillus pumilus* and *Bacillus subtilis*), Gram negative (*Shigella sonnei* and *Pseudomonas aeruginosa*) bacteria and third instar larvae of the dengue and Zika virus vector *Aedes aegypti*. The synthesized Ze-AuNPs were characterized by UV–vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The antibacterial assays testing Ze-AuNPs at 100 μg/ml showed that the zones of inhibition against Gram positive species *B. pumilus* and *B. subtilis* were 13.9 and 14.2 mm, respectively, while for Gram negative *S. sonnei* and *P. aeruginosa* they were 18.1 and 18.4 mm, respectively. Light and confocal laser scanning microscopy (CLSM) confirmed the interruption and disintegration of bacterial biofilm post-treatment with Ze-AuNPs at 100 μg/ml. In larvicidal assays on *A. aegypti*, $HAuCl_4$ and Ze-AuNPs treated third instar larvae of *A. aegypti* showed LC_{50} of 26.6 and 6.81 mg/L, respectively, and LC_{90} of 81.1 and 13.6 mg/L respectively. The histopathological analysis of *A. aegypti* treated with Ze-AuNPs showed complete disintegration of abdominal region, particularly the midgut and caeca, with loss of lateral and caudal hairs. The stereomicroscopic visualization of *A. aegypti* treated with Ze-AuNPs showed the loss of upper head hair, lower head hair, antenna hair, lateral hair and caudal hair. Overall, the study concludes that Ze-AuNPs have excellent antibacterial, antibiofilm effects and has ability to control the larval populations of *A. aegypti* mosquitoes.

1. Introduction

Zein comprises a complex mixture of storage proteins enriching maize seeds, where they were organized in protein bodies located in the endosperm. More than half of the amino acid residues of zein are nonpolar, which makes it one of the few natural proteins that can solubilize in aqueous ethanol solutions but not in water because of its high hydrophobicity [1]. Zein is considered as a natural, renewable, biodegradable and inexpensive biopolymer, which has been examined as a potential raw material for polymer application since 20th century. Recently, biopolymer studies and its application rose significantly in numerous scientific fields and various industries. Zein has been employed in numerous potential applications including emulsifiers [2], development and fabrication of several films, plastics, substrates for biomedical purposes [3], wheat substitute in gluten-free dough systems [4], drug delivery systems [5], food packaging and materials scaffolding for cell/tissue engineering [6]. Furthermore, zein possesses remarkable characteristics like compressibility, flexibility, high toughness, glossiness [7] and antioxidant properties [8].

Biofilms represent a protected mode of growth that allows

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microorganisms to survive in hostile environments. Biofilms play a major role in recalcitrant infections in the field of aquaculture and becomes difficult to eradicate, due to their drug-resistant phenotypes [9]. Mosquitoes are vectors of many diseases including filariasis, malaria, dengue, chikungunya and – more recently – Zika virus [52]. Viral diseases such as dengue, chikungunya and Zika virus are mostly vectored by *Aedes aegypti* (Diptera: Culicidae), which is a painful and persistent daytime biter [52]. Dengue causing hazards of around 2.5 million people that could be estimated by WHO and it is a major international health concern. However, it is essential to control mosquito population so that people can be protected from mosquito-borne diseases [10].

Currently, research on nanotechnology has gained increased attention because of the exceptional optical and biophysical properties of nanostructures [11]. Nanoparticles have expressed significant advances owing to a wide range of applications in the field of biomedicine, sensors, antimicrobials, antiparasitic drugs, catalysts, electronics, optical fibers, bio-labeling, and pest control [11–13]. Studies on the metallic nanoparticles have proved their antimicrobial potential. Green synthesized Au nanoparticles from *Nigella sativa* showed high antimicrobial effects against *Vibrio harveyi*[14], while chitosan-capped ZnO nano-composite has been found as effective on *Vibrio parahaemolyticus* and *Bacillus licheniformis*[15].

The large surface area of the nanoparticle enhances their interactions with the microbes to carry out broad-spectrum antimicrobial activities [16]. Gold is used to treat diseases such as small pox, skin ulcers and measles in the ancient times [17]. Currently, it is used in medical devices including pacemakers and gold plated stents for the management of heart diseases [18]. In the past few decades, several organo-gold complexes have emerged with promising antitumor, antimicrobial, antimalarial and anti-HIV activities [19]. Recently, several studies have reported natural polymers such as zein, chitosan, starch and tannic acid as reducing agents for the synthesis of gold and silver nanoparticles [20]. Therefore, the aim of the present study was to evaluate the antibacterial, antibiofilm and mosquito larvicidal activity of zein-capped Au nanoparticles (Ze-AuNPs) against both Gram positive (*B. pumilus* and *B. subtilis*) and Gram negative bacteria (*S. sonnei* and *P. aeruginosa*). Light and confocal laser scanning microscopy (CLSM) shed light on antibiofilm activity of Ze-AuNPs. Furthermore, larvicidal assays testing Ze-AuNPs on the Zika virus vector *A. aegypti* were carried out, followed by histopathological analyses of the effects of Ze-AuNPs on mosquito larvae. The synthesized Ze-AuNPs were characterized by UV–vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

2. Materials and Methods

2.1. Zein-mediated Gold Nanosynthesis and Related Nanocharacterization

A simple aqueous reduction method was used to synthesize gold nanoparticles using zein. Zein was purchased from Sigma Aldrich (USA). In this method, the zein polymer solution (2 g in 100 ml of 80% of aqueous ethanol) was prepared and stirred at room temperature [21]. 0.1698 g of chloroauric acid ($HAuCl_4$) was added to 500 ml distilled water. 50 ml of different concentrations (5, 10 and 15 ml) of zein solution were added to the chloroauric acid solution. After 10 min stirring, sodium borohydride was dropped slowly into above solution under vigorous stirring at 37 °C. The colloids were stirred than collected by centrifugation at 8000 x g (10 min). The pellet was washed with distilled water, dried at 80 °C in vacuum for 24 h and preserved in airtight vials [22].

The absorption spectrum of Ze-AuNPs was studied using UV–vis spectrophotometer (Shimadzu-UV-1800 Japan). The UV absorption was measured at different wavelengths ranging from 200 to 800 nm in diffuse reflectance mode using zein solution as reference at different time intervals. Ze-AuNPs were studied by XRD with X-ray diffractometer (PAN analytical XRD analyzer-X'pert PRO) at the acceleration voltage of 40 kV and a current 30 mA with Cu k radiation. X-ray diffraction spectrum of the Ze-AuNPs exhibited 2 theta values corresponding to the Au nanocrystal. FTIR spectrum was measured in the wave numbers range from 500 to 4000 cm^{-1} using FTIR spectrometer (Thermo Scientific Nicolet - iS5). The size and structure of synthesized Ze-AuNPs were determined by transmission electron microscope (JOEL model instrument 1200 EX) and scanning electron microscopy (SEM).

2.2. Culture of Bacteria Species

Gram positive (*B. pumilus* and *B. subtilis*) and Gram negative (*S. sonnei* and *P. aeruginosa*) bacteria were used from our laboratory culture collection. Bacterial cultures were transferred the test tubes containing nutrient broth (NB) and incubated without agitation for 24 h at 37 °C before testing.

2.3. A. aegypti Larval Rearing

The eggs of *A. aegypti* were procured from Indian Council of Medical Research (ICMR) Madurai (Tamil Nadu, India) using an O-type brush. Pathogen- and parasite-free *A. aegypti* was reared in laboratory conditions as described by [23].

2.4. Agar Well Diffusion Method for Antibacterial Assays

Antibacterial activity of Ze-AuNPs were screening against Gram positive (*B. pumilus* and *B. subtilis*) and Gram negative (*S. sonnei* and *P. aeruginosa*) bacteria using agar well diffusion assay. In this method, steriled Muller Hinton Agar (MHA) medium was poured onto Petri plates. All the bacterial culture was inoculated uniformly on MHA plates using sterile cotton swabs. About 6 mm diameter wells were created with the help of steriled cork borer for loading the Ze-AuNPs at different concentrations (5, 10, 15 and 20 μg/ml). After loading, the plates were allowed for incubation at 37 °C for 24 h. After 24 h, the zone of inhibition formed around the well was measured by a ruler in mm. Distilled water was used as control. The experiments were performed in triplicate.

2.5. Antibiofilm Activity

Biofilm inhibition of Gram positive (*B. pumilus* and *B. subtilis*) and Gram negative (*S. sonnei* and *P. aeruginosa*) bacteria was carried out by microtitre plate technique. Bacterial culture were allowed to grow on glass pieces (1 × 1 cm) placed in a 24 well plates containing 1.5 ml of nutrient broth loaded with different concentrations of Ze-AuNPs (25, 50, and 75 μg/ml). The plates were incubated for 3 days at 37 °C. After incubation, the glass pieces were washed twice with PBS followed by staining with crystal violet (0.4%) and then observed under a Nikon Microscope (ECLIPSE Ti 100 ×) at 40 × magnification. Independently, another set of glass pieces with biofilm grown as above were washed with PBS and stained with 0.1% of acridine orange. This biofilm formation was visualized under a Confocal laser scanning microscope (CLSM, Carl Zeiss LSM 710) using a 488 nm argon laser and band path 500–640 band pass emission filter and running Zen 2009 software (Carl Zeiss, Germany).

2.6. Mosquito Larvicidal Activity

The larvicidal activity was assessed by the standard procedure of [24]. In this assay, 3rd instar larvae were separated in five sets. Each set were provided with 6 larvae containing 50 ml of water. Different concentration of zein (20, 40, 60, 80, and 100 mg/ml), $HAuCl_4$ (20, 40, 60, 80, and 100 mg/ml) and Ze-AuNPs (5, 10, 15, 20, and 25 mg/ml) were introduced into the above setup. The control was setup with distilled