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Synergistic effect of epicatechin coated silver nanoparticles on antimicrobial activity of gentamicin against aspergillus niger

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Abstract

In present work, synergistic effect of epicatechin capped silver nanoparticles (ECAgNPs) with gentamicin was investigated in vitro and effect of ECAgNPs as a novel type of potential antimicrobial agent with gentamicin was observed.ECAgNPs with gentamicin showed remarkable antifungal activity when compared to the antifungal activities of epicatechin, gentamicin and silver nitrate. Gentamicin when combined with ECAgNPs revealed synergistic effect and this combination of ECAgNPs with gentamicin enhanced itsminimum inhibitory concentration (MIC) value that is, 78.3. When antifungal activity of gentamicin, epicatechin and silver nitrate was observed then it was noticed that epicatechin didn't possess antifungal effectiveness, whereas gentamicin and silver nitrate exhibited moderate antifungal effectiveness (MIC =37.9) and (MIC =35.4). Therefore, ECAgNPs inducedantifungal effectiveness when combined with gentamicin.

Keywords: *Silver nanoparticles; Gentamicin; Epicatechin; Epicatechin coated silver nanoparticles; antimicrobial agents; antifungal activity.*

1. Introduction

Nowadays intricate infections are treated through altered approaches and it is due to the absence of new antimicrobial drugs and as well as existence of drug resistance. Multidrug therapy and drug combination suggest the probability to overcome drug resistance[1]. It is fewer expected that pathogens produce resistance if drugs which affect varied and numerous molecular targets are used in combinations[2]. Multidrug therapy could comprise combinations of a number of antibiotics or of antibiotics with other antimicrobial agents.

Antibiotic effect of veterinary and human medication reduces when antimicrobial agents used extensively would contribute to the swift spread and development of bacterial resistance. [3]Resistance to antimicrobial agents in zoonotic enteropathogens (e.g. *Salmonellaspp.*), commensal bacteria (e.g. *Escherichia coli*) and animal pathogens (e.g. *Actinobacillus*spp. or *Pasteurella multocida*) has been stated[4-6].

Combination therapy is an exclusive approach to regulate bacterial infections in which antibiotics are given when combined with non-antimicrobial or antimicrobial agents. These adjuvants comprise of further non-antibiotic (such as cardiovascular medicines), antibiotics,resistance inhibitors like inhibitors of biofilm formation and β-lactamase inhibitors[7].Therefore the combination therapy that enhance the bioavailability and efficacy through two or more agents is basically a significant approach to overcome multi-drug resistance and to cure mixed diseases[8, 9].

Gentamicin is an aminoglycosidic antibioticwith a broad antibacterial spectrum[10] and has predominantly used to inhibit Gram-negative pathogens and tuberculosis[11].Gentamicin has lesser bioavailability after oral administration, as well as poor cellular diffusion that's why intravenous or intramuscular routes are valuable[12]. Gentamicin with AgNPs indicated highly effective combination and for *S.aureus,A. pleuropneumoniae* and *E.coli*, this combination is synergistic. In order to improve sensitivity of resistant A. pleuropneumoniae (MIC 8 µg/mL, breakpoint MIC ≥8 µg/mL) gentamicin was given in combination of AgNPs[13].

Epicatechin is a flavanol(flavan-3-ol) [14], and has occupied significant consideration owing to its numerous health benefits, ranging from weight loss to cancer[15]. Flavonoid intake reduce the possibility of cancer and coronary heart diseases[16, 17].

To the best of our knowledge, limited information is available regarding the effect of ECgNPs and gentamicin complex on the growth of aspergillus niger. Therefore, our work aimed to evaluate the effectiveness of combination therapy for this complex against *aspergillus niger*through *in vitro* experiment.

2. Materials and Methods:

Synthesis and characterization of ECAgNPs are discussed in detail in our previous publication[18]. To calculate minimum inhibitory concentration (MIC), the agar well diffusion method was employed. Determination of the MIC for gentamicin was measured with and without epicatechin coated AgNPs. PDA agar was used as medium to grow mycelial growth of fungus. Well of 5mm diameter with the help of cork borer was prepared at a corner of PDA poured plate containing penicillin 20,000 unit/L and streptomycin 200 mg/L. This freshly prepared PDA agar left to dry and then on this agar a disc of *Aspergillus niger* was placed separately in the center of the petri dish. The 100 µg/mL stock solutions of gentamicin, epicatechin and silver nitrate were used to observe antifungal activity. Each treatment was replicated 3 times. Petri dishes were incubated for 5 to 7 days at 28±2°C and zone of inhibition was measured in millimeter.

MIC formula

$I = (C-T)/C \times 100$

I= inhibition percentage

C= control

T =treated plates

The percent growth inhibition over control was determined according to the formula given[19].

3. Results and Discussion

Antimicrobial activity of ECAgNPs as a novel type of possible antimicrobial agent was investigated and synergistic effect of ECAgNPs with clinical drug was evaluated. Antifungalactivity of epicatechin, silver nitrate, gentamicin and ECAgNPs with gentamicin were observed *invitro* for the inhibition of human pathogenic fungi *Aspergillusniger* **(figure1)**.

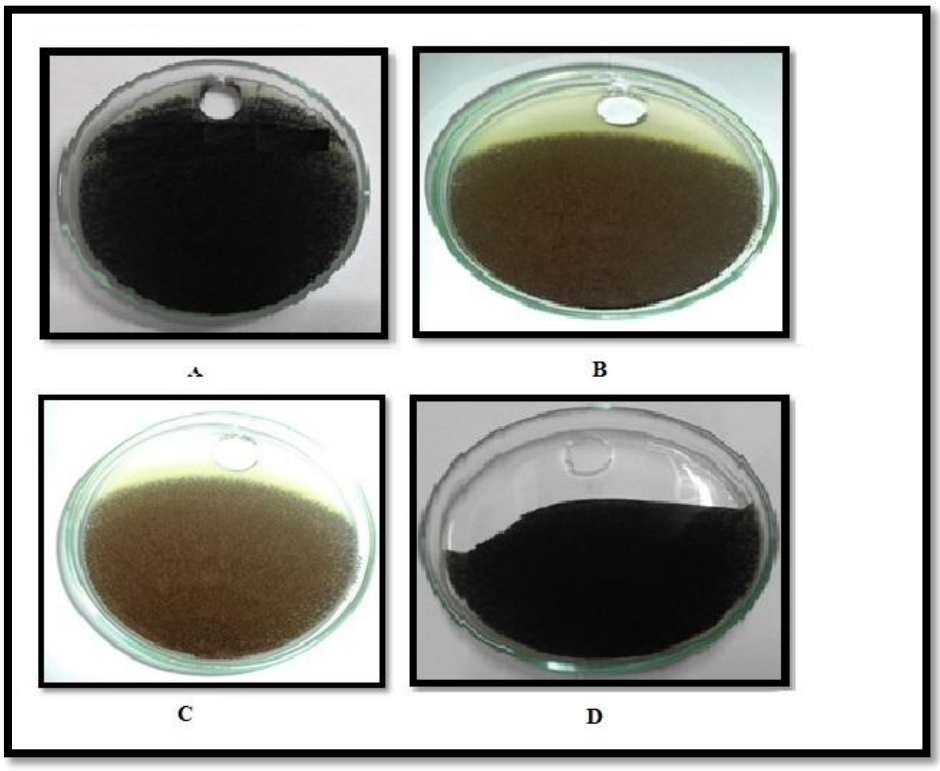

Figure 1. Effect on mycelial growth of *Aspergillus niger* by (a) epicatechin, (b) $AgNO_3$ (c) gentamicin (d) ECAgNPs with gentamicin.

The results of antibacterial activity indicate that combination of epicatechin coated silver nanoparticles with gentamicin showed strong synergistic effect **(table 1)**.

Table 1 *In vitro*, growth inhibitions of Aspergillus niger

Name	% MIC value
Gentamicin	37.9
$AgNO_3$	35.4
Epicatechin	0.0
AgNPs + gentamicin	78.3

Epicatechin coated silver nanoparticles with gentamicin showed remarkable antifungal activity when compared to the antifungal effectiveness of epicatechin, gentamicin and silver nitrate against tested *Aspergillus niger*. These results supported by percent MIC values examined for gentamicin and silver nitrate that were 37.9 and 35.4. Whereas combination of ECAgNPs with gentamicin indicated percent MIC of 78.3. So gentamicin and silver nitrate showed moderate antifungal activity, whereas in case of epicatechin antifungal activity was not observed.

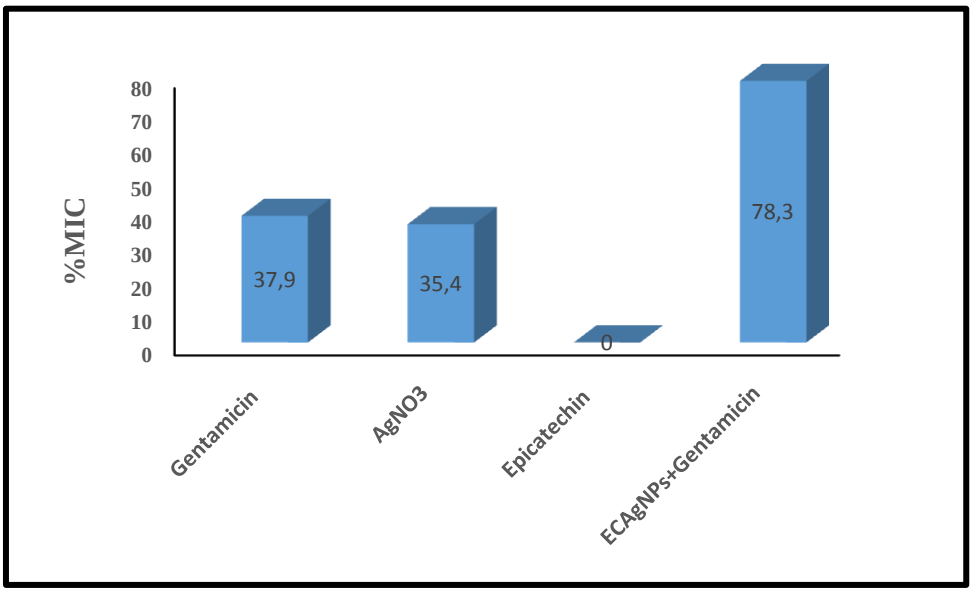

Figure 2. Percent minimum inhibitory concentration of gentamicin, AgNO3, epicatechin and ECAgNPs+gentamicin

Furthermore, ability of DNA to replicate was lost when microorganisms were treated with silver[20], which resulted in inactivated expression of various proteins and enzymes essential to ATP production[21]. Moreover, inhibition rate increased with the increment in concentration of silver nanoparticles, it is due to the fact that highly dense solution of silver nanoparticles can easily saturate and cohere to fungal hyphe and then inactivate plant pathogenic fungi[22].

Sodium deoxycholate (NaDC) silver nanoparticles suppressed the mycelia growth of *Colletotrichum gloeosporioides* which is the most destructive endophytic plant-pathogenic fungi[23, 24]. Silver nanoparticles in combination with gentamicin showed synergistic effectagainst *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*[25].

It was also shown that lesser concentrations of nano-sized silver would be satisfactory for microbial control because nanoparticles efficiently penetrate into microbial cells[26]. It is due to the high density at which solution cohere and saturate to fungal hyphe and eventuallyinactivate plant pathogenic fungi. When DNA is treated with silver then it loses its ability to replicate[27], resulting in inactivated expression of not only ribosomal subunit proteins but as well as certain other cellular proteins and enzymes essential to ATP production[28]. Moreover, it has also been hypothesized that silver chieflydisturbs the function of several enzymes, such as those in respiratory chain[29].

4. Conclusion

In conclusion, a novel type of ECAgNPs was used for the first time through an effective, easy and appropriate process. When compared with ECAgNPs and gentamicin alone, the ECAgNPs and gentamicin combination revealed considerably improved antifungal efficacy. Therefore, this combination of ECAgNPs with antimicrobial agents is definitely a favorable way to diminish the amount of antibiotics.

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