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ВЛИЯНИЕ НАНОЧАСТИЦ СЕРЕБРА НА БАКТЕРИЦИДНУЮ АКТИВНОСТЬ И АНТИБИОТИКОЧУВСТВИТЕЛЬНОСТЬ *SALMONELLA ENTERITIDIS* 182

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Проведены исследования по определению синергетического эффекта применения комбинаций антибактериальных веществ, включающих антибиотики, дезинфектант септабик и AgNPs. Выявлен значительный рост бактерицидной активности в комбинации септабик + AgNPs + нитокс и септабик + AgNPs + цефтиофур. Определение чувствительности *Salmonella enteritidis* 182 к антибактериальным препаратам показало наличие устойчивости к 8 (38,1%) препаратам, малой чувствительности – к 7 (33,3%), чувствительности – к 6 (28,6%) и отсутствие препаратов с высокой чувствительностью. После культивирования *S. enteritidis* 182 с антибактериальными препаратами и их комбинациями установлено увеличение количества лекарственных средств, к которым изучаемый штамм был чувствителен. Наличие чувствительности выявлено к 7–10 препаратам, что на 4,7–19,6% больше, чем в контрольных показателях. Установлена ранее отсутствовавшая высокая чувствительность к 2–8 (9,5–38,0%) антибактериальным препаратам. Культивирование *S. enteritidis* 182 с AgNPs показало наивысший рост антибиотикочувствительности из всех изучаемых комбинаций антибактериальных средств в виде увеличения размера задержки роста. Это дает основание предположить о ведущей роли AgNPs в преодолении антибиотикорезистентности. Инкубирование *S. enteritidis* 182 после контакта с септабиком и арговитом вызвало максимальное увеличение диаметра задержки роста микроорганизма без снижения показателя к отдельным видам препаратов (за исключением септабика, где установлена утрата чувствительности к тилозину). При добавлении различных антибиотиков к комбинации септабик + арговит отмечены факты снижения зоны задержки роста или его исчезновения. Описан комбинированный эффект сочетанного применения антибактериальных препаратов и наночастиц серебра в отношении бактерий с множественной лекарственной устойчивостью.

Ключевые слова: наночастицы серебра, *Salmonella enteritidis*, антибиотики, антибиотикорезистентность, AgNPs, септабик

EFFECT OF SILVER NANOPARTICLES ON BACTERICIDAL ACTIVITY AND ANTIBIOTIC SENSITIVITY OF *SALMONELLA ENTERITIDIS 182*

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Studies have been carried out to determine the synergetic effect of the use of combinations of antibacterial substances, including antibiotics, septabic disinfectant and AgNPs. A significant increase in bactericidal activity was revealed in the combination of septabic + AgNPs + nitox and septabic + AgNPs + ceftiofur. Determination of the sensitivity of *Salmonella enteritidis* 182 to antibacterial drugs showed the presence of resistance to 8 drugs (38.1%), low sensitivity to 7 (33.3%), sensitivity to 6 (28.6%) and the absence of preparations with high sensitivity indicators. After cultivation of *S. enteritidis* 182 with antibacterial drugs and their combinations, an increase in the number of preparations to which the studied strain was sensitive was found. The presence of sensitivity to 7–10 drugs was revealed, which is 4.7–19.6% higher than in the control indicators. A previously absent high sensitivity to 2–8 antibacterial drugs (9.5–38.0%) was established. The cultivation of *S. enteritidis* 182 with AgNPs showed the highest increase in antibiotic sensitivity of all the studied combinations of antibacterial agents in the form of the growth inhibition size increase. This suggests

a leading role of AgNPs in overcoming antibiotic resistance. Incubation of *S. enteritidis 182* after contact with septabic and argovite caused a maximum increase in the diameter of the growth inhibition of the microorganism without decreasing sensitivity to certain types of drugs (with the exception of septabic, where the loss of sensitivity to tylosin was established). When adding various antibiotics to the combination of septabic + argovit, a decrease in the growth inhibition zone or its disappearance were noted. The combined effect of the use of antibacterial drugs together with silver nanoparticles against bacteria with multidrug resistance is described.

Keywords: silver nanoparticles, *Salmonella enteritidis*, antibiotics, antibiotic resistance, AgNPs, septabik

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INTRODUCTION

The widespread use of antibacterial drugs in animal production has led to an increase in the isolation of antibiotic-resistant strains of microorganisms. This has significantly reduced the effectiveness of chemotherapy and has created conditions for the transmission of antibiotic-resistant genes through animal products to humans. In 2001 The UN WHO recognized the problem of antibiotic resistance as a global problem and developed a series of interventions to limit the occurrence and spread of this phenomenon, which equally affects both medical and veterinary service delivery. Carbapenem-resistant micro-organisms of the family Enterobacteriaceae are seen as a threat to public health. Bacterial strains isolated from cattle are a major channel for this resistance. Damages in the USA from infections caused by antibiotic-resistant microorganisms are estimated at $55-70 billion; similar losses in Europe exceed €1.5 billion per year[1, 2] [1].

A group of intestinal pathogens of the *Salmonella* genus, which can cause pathologies in animals and poultry and food poisoning in humans, pose a particular threat. Given the additional risks posed by persistent pathogens associated with the presence of antibiotic-resistant genes, the problem of infectious pathologies is becoming alarming and requires urgent solutions.

Research is underway to develop veterinary medicines that do not contain antibiotics but have antibacterial properties. One well-known method of treating and preventing infectious diseases is the use of silver preparations. These drugs are widely used in the treatment of gastrointestinal diseases of humans and animals, obstetric and gynecological (mastitis, endometritis) pathologies of cattle, as well as the treatment of wounds, ulcers, bedsores in surgical practice[3] [2-5]. In addition to the bactericidal properties of silver preparations, studies have revealed the presence of silver nanoparticles (AgNPs) to overcome and reduce the antibiotic

[1]World Health Organization. WHO Global Strategy for Containment of Antimicrobial Resistance. Geneva, 2001. WHO/CDS/CSR/DRS/2001.2.

[2]Strategy for preventing the spread of antimicrobial resistance: order of the Government of the Russian Federation No. 2045-r dated September 25, 2017.

[3]Blagitko E.M., Bugaychenko N.V., Shorina G.N. and others. Results of local application of argolite and hydropenta – silver-containing preparations on a natural mineral basis. Nanotechnologies and nanomaterials for biology and medicine: materials of scientific and practical research. conf. with int. participation: 2 hours. Novosibirsk, 2007. Part 2. P. 39–49.

resistance of opportunistic and pathogenic microflora[4]. Positive experience has been gained in the use of disinfectants as antibacterial medicines (ecocidal in oral administration), which opens up prospects for the use of these agents as therapeutic agents [6]. The need therefore arises to investigate the presence of possible synergistic properties in the combined use of antibacterial agents of different pharmacological groups.

A study of the effect of colloidal silver on the morphology and development of *Salmonella enteritidis* cell populations using scanning electron microscopy showed that AgNPs in low concentrations promotes partial destruction of the bacterial cell wall, prevents normal cell division, initiates the processes of heteromorphism. However, the S. enteritidis population remains viable. Once in a favorable environment, it fully restores its morphological properties and the possibility of growth and development (bacteriostatic effect). At high concentrations, AgNPs causes rapid cell death in the population. Thus, the bactericidal effect of colloidal silver solutions depends directly on the concentration of nanoparticles in them. The higher their concentration, the deeper the lesion of cellular structures, the more pronounced is the disinfecting effect of the applied preparation [7].

It was found that nanosized systems can not only improve the therapeutic activity of antibacterial agents, but also inhibit the stimulation of resistance by overcoming resistance strategies developed by bacteria, including the degradation of the drug under the action of β-lactamase, thickening the walls of bacterial cells [8]. The synergistic effect of AgNPs in combination with gentamicin against *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* showed a significant increase in the diameter (up to 26-34 mm) of bacterial growth retention zones [9].

The aim of the study was to investigate the bactericidal properties of various combinations of antibacterial agents and their effect on the possibility of overcoming antibiotic resistance in *S. enteritidis* 182.

MATERIAL AND METHODS

A preparation argovit containing AgNPs (12-14 μg/ml) was used for the study; ceftimag, containing ceftiofur hydrochloride 100 mg as an active substance in 1 ml; containing methyl ether of paraoxybenzoic acid 1.8 mg, propyl ether of paraoxybenzoic acid 0.2 mg and propylene glycol dicaprylate/dicaprates up to 1 ml; oxytetracycline as a 10% aqueous solution; enrofloxacin containing 50 mg of enrofloxacin in 1 ml; gentamicin, containing 40 mg of gentamicin sulfate in 1 ml; nitox, containing 200 mg of oxytetracycline dihydrate as an active ingredient in 1 ml of the formulation; excipients - magnesium oxide, N,N-dimethylacetamide, rongalit, monoethanolamine and water for injection; azithromycin in the form of azithromycin citrate derived from azithromycin dihydrate, 100 mg in 1 ml; excipients - citric acid monohydrate, sodium hydroxide, water for injection.

The disinfectant Septabic contains the active ingredient urea clathrate didecyldimethylammonium bromide, which is active against Gram-positive and Gram-negative bacteria (including Mycobacterium tuberculosis), dermatophytes, yeast-like fungi, candida, hepatitis B virus, HIV, influenza viruses, parainfluenza[5].

Microbial sensitivity of the *S. enteritidis* ATCC 182 reference strain to antibacterial agents and combinations was determined from a dilution with the lowest bacteriostatic concentration, 0.2 ml of which was added to an AMP and the antibiotic sensitivity of the microorganisms was determined by discodiffusion.

Determination of sensitivity was carried out to 21 antibacterial drugs with subsequent incubation for 24 hours at a temperature of 37.5 ± 0.5 0 C.The sensitivity of microorganisms to antibiotics was determined by the degree of delay in the growth diameter around the disc:

[4]Mamonova I.A. The influence of nanoparticles of the transition group of metals on antibiotic-resistant strains of microorganisms: author. dis. PhD in biology. M., 2013. 21 p.

[5]https://infodez.ru/product/1452_septabik.html

up to 10 mm - resistant, up to 15 - insensitive, up to 20 - sensitive, more than 20 mm - highly sensitive[6].

RESULTS AND DISCUSSION

Determination of the bactericidal activity of antibacterial agents in combination with AgNPs and septabic disinfectant is justified by its possible use in the treatment of infectious pathologies due to acceptable parameters of acute toxicity, which refers to class 3 (GOST 12.1.007-76) of moderately hazardous compound when introduced in the stomach and to class 4 of low-hazardous compound when applied to skin and when inhaled at saturation concentration. AgNPs has a mild sensitising effect[7].

Studies carried out to determine the synergistic effect of combinations of antibacterial agents including antibiotics, disinfectant preparation and AgNPs showed a significant increase in bactericidal activity in the combination septabic + AgNPs + nitox and septabic + AgNPs + ceftiofur (see Table 1).

Determination of the sensitivity of *S. enteritidis* 182 to antibacterial agents showed resistance to 8 (38.1%), low sensitivity to 7 (33.3%), sensitivity to 6 (28.6%) and no drugs with high sensitivity. After culturing *S. enteritidis* 182 with antibacterial agents and their combinations an increase in the number of drugs to which the studied strain was sensitive was found. The presence of sensitivity was detected to 7-10 drugs, which is 4.7-19.6% more than in the target scores. A previously absent high sensitivity to 2–8 (9.5–38.0%) antibacterial drugs was established (see Table 2).

The cultivation of *S. enteritidis* 182 with AgNPs showed the highest increase in antibiotic sensitivity among all the studied combinations of antibacterial agents in the form of the largest growth retardation of the microorganism, which suggests the leading role of AgNPs in overcoming antibiotic resistance. Incubation of *S. enteritidis* 182 after contact with septabic and argovit caused a maximum increase in the diameter of the growth retardation of the microorganism without a decrease in the indicator for certain types of drugs (with the exception of septabic, where the loss of sensitivity to tylosin was established). With the addition of various antibiotics to the combination of septabic + argovit, facts of a decrease in the growth retardation zone or its disappearance were noted. Thus, enrofloxacin reduced the diameter of the growth retardation of a microorganism or led to its disappearance by 6 (28.6%) drugs, oxytetracycline, gentamicin - by 4 (19.0%), nitox, ceftiofur, azithromycin - by 2 (9.5%) ... In these cases, in all combinations, a loss of sensitivity to tylosin and neomycin was noted when azithromycin, enrofloxacin, nitox and ceftiofur were added to the combination of septabic + argovit (see Tables 3, 4).

The results of the conducted studies indicate the presence of synergistic properties of the combined use of antibacterial drugs and confirm previous studies by other authors. The

Табл. 1. Чувствительность *S. enteritidis* 182 к антибактериальным препаратам и их комбинациям

Table 1. Sensitivity of *S. enteritidis* 182 to antibacterial drugs and their combinations

Preparation	Concentration, μg/ml		
	AgNPs	Septabic	Antibiotic
AgNPs	115	–	–
Septabic	–	312	–
Septabic + AgNPs	0,2	156	–
Septabic + AgNPs + oxytetracycline	0,1	0,78	0,78
Septabic + AgNPs + azithromycin	0,05	0,39	0,39
Septabic +AgNPs + gentamicin	0,05	0,39	0,39
Septabic + AgNPs + enrofloxacin	0,05	0,39	0,39
Septabic + AgNPs + nitox	0,00125	0,0975	0,0975
Septabic + AgNPs + ceftiofur	0,00125	0,0975	0,0975

[6]Determination of the sensitivity of microorganisms to antibacterial drugs: guidelines MUK 4.2. 1890-04, CRIE. M., 2004.101 p.
[7]https://infodez.ru/product/1452_septabik.html

combined effect of the combined use of antibacterial drugs and silver nanoparticles against bacteria with multidrug resistance is described. It was found that the synergistic effect of the antibiotics such as ciprofloxacin, imipenem, gentamicin, vancomycin, trimethoprim and nanoparticles led to an increase in antibacterial activity 0.2-7.0 times (on average 2.8). This shows that nanoparticles can be effectively used in combination with antibiotics to increase their effectiveness against various pathogenic microorganisms [10]. The antibacterial effect of the combined use of AgNPs and vancomycin against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumonia* has been established [11].

Synergistic antibacterial activity in combination of AgNPs with tetracycline, neomycin and penicillin was tested against antibiotic-resistant *S. typhimurium* DT104. A dose-dependent inhibition of the growth of bacteria *S. typhimurium* DT104 was noted for the complexes tetracycline - AgNPs and neomycin - AgNPs (0.07 and 0.43 μg / ml, respectively). At the same time, the combination of penicillin - AgNPs did not inhibit the growth of the microorganism [12]. The antimicrobial activity of the synthesized bioconjugates AgNPs and endogenous antibiotics was studied. Conjugates of AgNPs with peptide C-Bac3.4 have antimicrobial activity, including against the antibiotic-resistant *P. aeruginosa* strain, as well as the methicillin-resistant *St. aureus* ATCC 33591. Studies have shown that the complexes of AgNPs with antimicrobial peptides do not exhibit the pronounced membranolytic effect inherent in peptides [13].

AgNPs, synthesized by *A. calcoaceticus* LRVP54 for 24 h, in the form of monodisperse spherical nanoparticles with a size of 8–12 nm, were obtained from metal salts of silver nitrate 0.7 mm in size with subsequent heating at a temperature of 70 °C. The disc diffusion method established a higher antibacterial activity against gram-negative microorganisms. A high synergistic antibacterial effect (3.8 times) of the use of AgNPs and vancomycin for *Enterobacter aerogenes* was established, while a decrease in the minimum bactericidal concentration was observed. It is noted that multidrug-resistant *Acinetobacter baumannii* is highly sensitive in the presence of AgNPs and to antibiotics, with the exception of cephalosporins. A similar effect was observed when AgNPs were added to the culture broth with vancomycin-resistant *Streptococcus mutans*. Thus, biogenically synthesized AgNPs showed a significant synergistic antibacterial effect on β-lactam antibiotics [14].

It has been found that the combination of silver and sulfadimethoxine makes it possible to increase the antibacterial activity and create a drug to which bacteria are not addictive at certain concentrations of silver in solution. Preparations with AgNPs and sulfadimethox-

Табл. 2. Изменение чувствительности *S. enteritidis* 182 после контакта с антибактериальными средствами и их комбинациями

Table 2. Changes in the sensitivity of *S. enteritidis* 182 after contact with antibacterial agents and their combinations

Preparation	Number of preparations							
	У	%	М	%	Ч	%	В	%
Argovit	1	4,8	3	14,3	12	57,1	5	23,8
Septabic	4	19,0	2	9,5	10	47,6	5	23,8
Septabic + AgNPs	3	14,3	2	9,5	10	47,6	6	28,6
Septabic + AgNPs + oxytetracycline	8	38,0	3	14,3	7	33,3	3	14,3
Septabic + AgNPs + azithromycin	3	14,3	2	9,5	8	38,0	8	38,0
Septabic + AgNPs + gentamicin	7	33,3	2	9,5	9	42,74	3	14,3
Septabic + AgNPs + enrofloxacin	12	57,0	–	–	7	33,3	2	9,5
Septabic + AgNPs + nitox	4	19,0	1	4,75	10	47,6	6	28,6
Septabic + AgNPs + ceftiofur	6	28,6	3	14,3	8	38,0	4	19,0

Note. Y - stable, M - insensitive, H - sensitive, V - highly sensitive.

Табл. 3. Изменение зоны задержки роста *S. enteritidis* 182 после контакта с антибактериальными препаратами и их комбинациями

Table 3. Changes in the growth inhibition zone of *S. enteritidis* 182 after contact with antibacterial drugs and their combinations

Preparation	Control group	Septabic	%	Septabic + AgNPs	%	Septabic + AgNPs + oxytetracycline	%	Septabic + AgNPs + azithromycin	%	Septabic + AgNPs + gentamicin	%
Ampicillin / sulfabactam	–	–	–	21	100	–	–	11	100	–	–
Ampicillin	15	20	33,3	22	46,7	14	–6,7	21	40	–	–100
Amikacin	–	21	100	20	100	20	100	22	100	20	100
Benzylpenicillin	–	–	–	19	100	–	–	–	–	–	–
Gentamicin	15	18	20	16	6,7	20	33,3	20	33,3	20	33,3
Doxycycline	15	13	–13,3	16	6,7	–	–100	16	6,7	13	–13,3
Polymyxin	-	15	100	19	100	–	–	15	100	15	100
Carbecillin	20	21	5	21	5	21	5	25	25	23	15
Norfloxacin	–	24	100	–	–	23	100	21	100	22	100
Neomycin	16	18	12,5	18	12,5	18	12,5	–	–100	20	25
Enrofloxacin	17	18	5,9	18	5,9	20	17,6	22	29,4	20	17,6
Ciprofloxacin	20	25	25	23	100	21	5	21	5	25	25
Tetracycline	–	20	100	15	100	14	100	18	100	17	100
Oxytetracycline	15	18	20	13	13,3	13	–13,3	17	13,3	18	20
Lincomycin	–	16	100	–	–	–	–	20	100	–	–
Tylosin	15	–	–100	21	27,0	–	–100	–	-100	–	–100
Levomycetin	15	20	33,3	16	6,7	16	6,7	22	46,7	20	33,3
Streptomycin	15	20	33,3	18	20	20	33,3	21	40	16	6,7
Ticarcillin / clavulanic acid	19	20	5,3	24	26,3	16	–15,8	20	5,3	–	–100
Ofloxacin	16	23	43,7	17	6,2	–	–100	20	25	17	6,2
Rifampicin	–	–	–	–	–	–	–	20	100	–	–

ine have less antibacterial activity compared to complexes of silver in the ionic form, but the antimicrobial activity of preparations with AgNPs becomes less dependent on the concentration and nature of the substrate[8].

The synergistic and antagonistic effects of the combined use of metal nanoparticles and antibiotics against pathogenic strains of microorganisms are noted. AgNPs and ZnONPs exhibited increased antibacterial activity with increasing concentration against *S. aureus*, *E. coli*. The synergistic effect of the antibiotics such as azithromycin, cefotaxime, cefuroxime, fosfomycin and chloramphenicol against *E. coli* and *S. aureus* was significantly higher in the presence of AgNPs compared with antibiotic use alone (see footnote 8).

The synergistic effect of the antibiotics such as azithromycin, oxacillin, cefotaxime, cefuroxime, fosfomycin, and oxytetracycline against *E. coli* was significantly higher in the presence of ZnONPs compared with the use of the antibiotic alone. The synergistic effect of the antibiotics azithromycin, cefotoxime, cefofoxime, cefosfimoxime, cefimpoxim, cefofoxime, cefosfimoxime, cefoximol, and oxytetracycline against *S. aureus* was also significantly higher in the presence of ZnONPs compared to monouse of antibiotics [15].

[8] Lopanov A.N., Lysykh V.A. Antibacterial activity of sulfadimethoxine with silver. Perspective scientific research: experience, problems and development prospects: materials of the international scientific conference. Ufa, 2020, pp. 93–100.

Табл. 4. Изменение зоны задержки роста *S. enteritidis* 182 после контакта с антибактериальными препаратами и их комбинациями

Table 4. Changes in the growth inhibition zone of *S. enteritidis* 182 after contact with antibacterial drugs and their combinations

Preparation	Control group	Argo-vit	%	Septabic + Ag + enrofloxacin	%	Septabic + AgNPs+ nitox	%	Septabic + AgNPs + ceftiofur	%
Ampicillin / sulfabactam	–	12	100	–	–	13	100	13	100
Ampicillin	15	21		–	–100	22	46,7	20	33,3
Amikacin	–	20	100	–	–	20	100	21	100
Benzylpenicillin	–	11	100	–	–	16	100	–	–
Gentamicin	15	20	33,3	22	46,7	24	60	19	26,7
Doxycycline	15	16	6,7	16	6,7	16	6,7	13	–13,3
Polymyxin	–	16	100	–	–	20	100	16	100
Carbecillin	20	23	15	–	–100	23	15	–	–20
Norfloxacin	–	21	100	22	100	25	100	20	100
Neomycin	16	18	12,5	–	–100	–	–100	13	–18,7
Enrofloxacin	17	21	23,5	20	–17,6	20	17,6	22	29,4
Ciprofloxacin	20	21	5	–	–100	23	15	23	15
Tetracycline	–	20	100	–	–	20	100	–	–
Oxytetracycline	15	18	20	20	33,3	20	33,3	16	6,7
Lincomycin	–	–	–	–	–	–	–	–	–
Tylosin	15	16	6,7	–	–100	–	–100	–	–100
Levomycetin	15	16	6,7	20	33,3	20	33,3	16	6,7
Streptomycin	15	20	33,3	20	33,3	20	33,3	20	33,3
Ticarcillin / clavulanic acid	19	20	5,3	20	5,3	22	15,8	25	31,6
Ofloxacin	16	18	12,5	19	18,7	18	12,5	18	12,5
Rifampicin	–	10	100	–	–	10	100	–	–

Literature data confirm the different antibacterial effect of using AgNPs and antibiotics against bacteria isolated from animals that exhibit resistance to antibiotics. The inhibitory concentration was calculated to classify the observed collective antibacterial activity as synergistic, additive (only the sum of individual drug effects), indifferent (no effect), or antagonistic. Of the 40 tests performed, 7 were synergistic, 17 were additive and 16 were indifferent. None of the combinations tested showed an antagonistic effect. Most synergistic effects were observed for combinations with AgNPs co-administered with gentamicin. The greatest increase in antibacterial activity was found in combination therapy with penicillin G against *Actinobacillus pleuropneumoniae*, *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*. Initially resistant to amoxicillin, gentamicin and colistin, they are susceptible to these antibiotics in combination with AgNPs. Research shows that AgNPs have potential as adjuvants for the treatment of bacterial diseases in animals [16].

Synergistic action of AgNPs in combination with erythromycin and levofloxacin against *St. aureus* was observed. Antimicrobial activity with antibiotics compared to pure silver nanoparticles was increased by a factor of 1.16-1.32. This synergism may be relevant for the treatment of infections caused by multidrug-resistant bacteria [17].

Analysis of the research results of many authors shows both a marked increase in the bactericidal activity of antibiotics when combined with AgNPs and its decrease. Given the variety of antibacterial drugs used in medicine and agriculture, this mixed result requires further research in the search for new combinations with AgNPs.

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