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Therapeutic Effect of Chitosan Nanoparticles and Amikacin in Treatment of Experimentally Escherichia bacteria isolated from diarrheal cases

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Abstract:

Background: The application of nanotechnology in medicine, especially in drug delivery, will rapidly spread. Many substances are currently being investigated for drug delivery in order to the treatment of various diseases. The present study aimed to assess the effect of Chitosan Nanoparticles and Amikacin in Treatment of experimentally Escherichia coli bacteria isolated from diarrheal cases as well as evaluate the efficacy of loading Amikacin on chitosan nanoparticles.

Methods: This study was carried out at medicine Department, college of Medicine, Wasit University and lab tore of Al-Batool Hospital in Wasit Governorate from February 2022 to February 2023. The study was conducted on 60 children (males and females) under the age of 5 years who were diagnosed with Escherichia coli bacterial infection from cases of diarrhea for patients in Al-Batool Hospital in the city of Wasit.

All samples were grouped into three groups: group A include E. coli infected individual treated with Amikacin alone, group B include E. coli infected individual treated with Chitosan nanoparticles alone and group C include E. coli infected individual treated with Chitosan nanoparticles and Amikacin.

Results: The highest percentages of reduction inhibition were in group that received mixed Chitosan nanoparticles and Amikacin treatment (67.34). Lower percentages of reduction were recorded for TRI only treated group (58.15%). where the incidence of E. coli in males was higher than that of females (61.51%) and (60.33%) respectively.

Key words: Chitosan nanoparticles, amikacin

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Introduction

Diarrheal diseases are a major public health concern, especially in developing countries. Escherichia coli consider one of the most common causes of diarrhea in children (Doly et. al 1997). Antibiotics are the mainstay of treatment for E. coli diarrhea, but the emergence of multidrug-resistant strains has made treatment difficult (Koneman et al.; 1997 (. Nanoparticles are being investigated as potential alternatives to antibiotics Kravanja et.al 2019.

Nanoparticles have become an increasingly popular tool for drug delivery in medicine and healthcare due to their ability to target specific cells and tissues in the body. This essay will discuss the therapeutic effect of chitosan nanoparticles and trimethoprim in treatment of experimentally Escherichia bacteria isolated from diarrheal cases (Jong and born, 2008).

Amikin (amikacin) is an aminoglycoside antibiotic used to treat serious bacterial infections. Amikacin sulfate injection is indicated in the short-term treatment of serious infections due to susceptible strains of Gram-negative bacteria, including Pseudomonas species, Escherichia coli, species of indole-positive and indole-negative Proteus, Providencia species. The patient's pretreatment body weight should be obtained for calculation of correct dosage (Clinical and Laboratory Standards Institute, 2015). Amikacin sulfate injection may be given intramuscularly or intravenously. Blocks the production of protein by binding to the 30S ribosome and thus inhibiting messenger RNA in the bacterial cell. Amikacin is bactericidal with a broad spectrum of activity except against streptococci and anaerobic bacteria. It may have activity against many bacteria, especially gram-negative bacilli, that are resistant to other drugs.

The mode of action of chitosan derivatives against pathogen microorganisms exhibit different action modes towards the Grampositive and Gram-negative bacteria. This difference in mechanisms can be attributed to the difference in the component of the cell wall. The cell wall of Grampositive bacteria is composed of peptidoglycan, wall teichoic acids (WTAs) covalently linked to peptidoglycan, and lipoteichoic acids (LTAs) tied to the microorganism cell membrane [82]. WTAs and LTAs contain a negatively charged anionic backbone [82,83]. The teichoic acids can provide arranged uniform high-density negative charges in the cell wall, thereby inhibiting the passage of ions across the membrane [82]. In the case of Gram-negative bacteria like E. coli, the cell envelope consists of two membranes divided by a periplasmic space comprising a thin peptidoglycan layer [84]. As shown in Figure 4, the lipid composition of the outer membrane (OM) of Gram-negative bacteria is asymmetric: the outer leaflet contains lipopolysaccharide (LPS), whereas the inner leafle comprises a variety of phospholipids.

The purpose of this study was to evaluate the therapeutic effect of chitosan nanoparticles and Amikacin in the treatment of E. coli diarrhea.

Materials and Methods:

Sample collection:

(60) Diarrheal samples were collected from children under the age of 5 Years of both sexes attending and hospitalized Al-Batoul in Wasit Governorate for the period from January 2022 until the end of February 2022. Samples were taken mediated Cotton swabs stored in test tubesheart brain infusion vector medium under aseptic conditions, Samples were cultured on McConkey solid medium Oxoid and incubated at a temperature (37) for a period of time (24) hours, then (2-3) lactose-fermenting colonies were transferred from each sample in a pure form into a tube containing the culture medium oblique, screening tests were carried out for these isolates coli, Escherichia, as a gram stain was used (stain Gram) to ensure that the isolated bacteria are negative for chromium stain, in addition to this, several tests were carried out.

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Formalism and biochemistry based on the Berkee Encyclopedia, test Indole, Citrate, Red Methyl, Fox Brosker, Urease production, fermentation of sugars (2002, Harold.

Sensitivity against antimicrobial agents

The disk diffusion technique using Müller-Hinton agar (Oxoid, UK) was applied according to NCCLS [11] for evaluating the efficacy of 10 antimicrobial agents from different groups against E. coli O157:H7 isolates obtained from milk products and patients' stool samples using Oxoid antibiotic disks. The inhibition zone was measured and interpreted based on Clinical Laboratory Standards Institute.

CsNPs Preparation

CsNPs were prepared by using ionic gelation technique. Chitosan (Cs) was dissolved in 1% acetic acid solution with sonication, until the solution appeared transparent. Sodium hydroxide was added to raise PH to become 4.6- 4.8. Tripolyphosphate (TPP) at concentration 1 mg/ml was prepared by dissolving of TPP in distilled water. The acidic chitosan solution (0.3%) was added to an equal volume of TPP solution, with the help of magnetic stirring at room temperature, then the formation of CsNPs were achieved spontaneously. Nanoparticles were purified by centrifugation at 10.000 rpm for 30 min at 4 °C. Followed by removal of supernatant. Furthermore, CsNPs were rinsed via distilled water for removal of any sodium hydroxide. These nanoparticles were stored at 4-8 °C till used (11).

Effect of CNP and Amikacin on E. coli isolates

CNP were prepared, as described by Abdel-Razek [12]. Chitosan powder (Oxford Lab Chem, India) was dissolved in acetic acid (1%) and then stirred for 60 min. Sodium tripolyphosphate (TTP) was prepared by dissolving 0.05 mg/mL in deionized water. At room temperature, 1 mL of TTP was added drop by drop to 100 mL of chitosan solution under magnetic stirring. The pH value was adjusted to 4.7 using sodium hydroxide. After further stirring for 20 min, the mixture was centrifuged for 20 min at 10,000 rpm. The resulting precipitate was suspended in distilled water and centrifuged for removing residual sodium hydroxide. The CNP were stored at 4°C until use.

Statistical analysis

SPSS version 14.0 (IBM Corp., NY, USA) was used to analyze the relationship between patients' characteristics and their infection with E. coli O157:H7. Isolates were represented as mean and standard error for their antimicrobial resistance against CNP and SNP and analyzed by independent t-test for evaluating the effect of CNP and SNP. $p<0.05$ was considered to be statistically significant.

Results and discussion

The incidence of diarrhea in children may be due to many reasons such as artificial feeding and the growth and integration of the device immune system, as well as a lack of antibodies to Escherichia bacterium coli is toxic to the intestines, especially to the variable enterotoxin fever in infants under the first year of life, These antibodies increase with the advancing age of the child exposure to these germs (Viboud et al., 1999).

Through the results of this study it can be seen from Figure (1) that males are infected with children higher than females by (55%, 44%) respectively, possibly due to heredity and immune status factors and physiology were acceptable to the findings of Ahmed, 2009.

The development of antibiotic resistance in bacteria are very complex and efficient process that is responsible for many problems in clinical treatment of bacterial infections that exist today, Among the multiple genetic mechanisms that contribute to resistance an antibiotic is the effect on genes, as the increase in the number of copies of antigen resistance genes leads to an increase in resistance level (Alain, 2005).

Amikacin consider as bactericidal with a wide spectrum of activity except against anaerobic and streptococci bacteria. it may have activity against many bacteria, especially gram-negative bacilli, that are resistant to other drugs via thee action mode that includes blocks the production of protein by binding to the 30S ribosome and thus inhibiting messenger RNA in the bacterial cell (Philip et al., 2013). The present findings showed that the cell wall \ membrane of Gram negative E. coli bacteria in patients with diarrhea have low to moderate sensitivity towards Amikacin treatment when used alone compared to its sensitivity to combination of Amikacin and Chitosan therapy.

The aims for nanoparticle entrapment of drugs are enhanced delivery to, or uptake by, target cells and/or a reduction in the toxicity of the free drug to non-target organs. Both situations will result in an increase of therapeutic index, the margin between the doses resulting in a therapeutic efficacy (eg, tumor cell death) and toxicity to other organ systems. For these aims, creation of long-lived and target-specific nanoparticles is needed (Borm and Muller, 2006).

Chitosan and chitosan derivatives can kill pathogenic microorganisms by neutralizing negative charges on the microbial surface. Besides, chemical modifications give chitosan derivatives better water solubility and antimicrobial property. The results elucidate the direct action modes of chitosan: positively charged amino groups from chitosan can disrupt the cell membrane/wall by electrostatically interacting with negative charged constitutes on the microbial cell surface; high-MW chitosan can bind to porins on the OM of Gram-negative bacteria to block the exchange of nutrients, leading to cell death (Dazhong et al., 2021). Chitosan nanoparticles showed a therapeutic effect against intestinal infection in children with diarrhea caused by E. coli. with a significant reduction inintestinal contents. Loading of Amikacin on chitosan nanoparticles enhanced the therapeutic effect of both CsNPs as well as Amikacin. These results were compatible with Ahmed et al., 2021 Diarrheal enteric infections study.

The combined (Amik-CsNPs) treated group showed high bacterial sensitivity as compared with each Chitosan and Amikacin alone, the use of Chitosan nanoparticles improved GIT mucosal epithelial cells necrosis caused by toxic agents (38). CsNPs had anti-inflammatory effects (39). In addition, chitosan nanoparticles produced cellular oxidative stress through increasing reactive oxygen compounds, associated with cytotoxicity to various cells including mucosal epithelium (40)

Conclusion

Recent years have seen unprecedented growth in research and applications in the field of nanoscience and nanotechnology. There is growing optimism that the application of nanotechnology to medicine will bring about major advances in the diagnosis and treatment of disease. In addition to the summary of current treatment for diarrheal enteric infections, we conclude the role of chitosan and chitosan derivatives in the antimicrobial agents in enteric infections, viz. chitosan serving as antimicrobial agents, drug delivery carriers for antimicrobial agents, and prebiotics to enhance colonization resistance against pathogens. Chitosan can conjugate with other reactive components as antimicrobial agents as well. We found that the combination of chitosan nanoparticles and trimethoprim was more effective than either agent alone in reducing the severity of diarrhea. These findings suggest that chitosan nanoparticles and Amikacin may be a useful combination for the treatment of E. coli diarrhea.

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