

Effect of green tea on the effectiveness of Cefixime on *Escherichia coli* and *Pseudomonas aeruginosa* bacteria

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Received 13/10/2010 Accepted 24/4/2011

Abstract

Cefixime is a broad-spectrum oral cephalosporin with a long half-life and has a bactericidal effect against both gram-positive and gram-negative bacteria. In this study the effects of cefixime on two types of bacteria, was compared to its effect after adding phenolic extracts of green tea *Camella sinenses*. The susceptibility test was used to study the effect of drug cefixime in the growth of two types of bacteria is *Escherichia coli*, *Pseudomonas aeruginosa*, and was extracted the phenoles from the green tea powder and study their effect in the growth of bacterial species under study, and then added these phenolic extracts to drug cefixime and studied its effect on the growth of bacterial species under study after the addition. Cefixime showed high inhibitory effect on the growth of bacterial species under study before adding the phenolic extracts and the drug was less influential in the growth of bacteria after the addition, while the phenolic extracts before addition showed different activities in inhibiting the growth of these types of bacteria. Add- process has led to the case of antagonism between the phenolic extracts and drug cefixime.

تأثير الشاي الاخضر على فعالية مضاد السيفكسيم على بكتريا أي كولاي
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المستخلص

السيفكسيم مضاد واسع الطيف من مجموعة السيفالوسبورين ذو مفعول طويل الأمد وهو عامل قاتل لكل من الجراثيم السالبة والموجبة الغرام. استخدم اختبار الحساسية لدراسة تأثير عقار السيفكسيم في نمو نوعين من البكتريا وهي *Escherichia coli*، *Pseudomonas aeruginosa*، وتم استخلاص الفينولات من مسحوق الشاي الأخضر ودراسة تأثيرها في نمو الأنواع البكتيرية قيد الدراسة ثم أضيفت هذه المستخلصات الفينولية إلى عقار السيفكسيم ودرس تأثيره في نمو هذين النوعين من البكتريا بعد الإضافة. أظهر عقار السيفكسيم تأثيرا تثبيطيا عاليا في نمو الأنواع البكتيرية قيد الدراسة قبل إضافة المستخلصات الفينولية في حين كان العقار اقل تأثيرا في نمو البكتريا بعد الإضافة. بينما أظهرت المستخلصات الفينولية فعاليات مختلفة في تثبيط نمو هذين النوعين من البكتريا قبل الإضافة. إن عملية الإضافة هذه قد أدت إلى حالة تضاد بين كل من المستخلصات الفينولية وعقار السيفكسيم.

Introduction

Cefixime is an antibiotic in a class of drugs called cephalosporin. It is a third-generation cephalosporin available in an oral formulation⁽¹⁾ and has pharmacokinetic activity suitable for single-dose administration.⁽²⁾ cefixime is an effective orally active cephalosporin with a relatively long elimination half-life permitting a simplified treatment regimen.⁽³⁾ cefixime is commonly used in the treatment of otitis media, respiratory tract infections,^(4,5) and urinary tract infections caused by susceptible organisms.⁽³⁾ Recent clinical studies have shown the excellent efficacy of cefixime for the treatment of typhoid fever.^(6,7) . The microorganisms cause many diseases, whether skin, gastrointestinal or respiratory infections and other as *Pseudomonas aeruginosa* and *Escherichia coli*,^(8,9) and there are some bacteria appear successive resistance with time for most of the antibiotics⁽¹⁰⁾, which is make a troubles in the treatment of infection with these bacteria. The fact that medicinal plants are safe to use without adverse effects, and contain a lot of chemical compounds called effective material as a result of its effectiveness against many diseases as phenolic compound which is found in a green tea commonly used. Therefore has been extracted and added to the drug cefixime and study the effect of this drug before and after adding the phenolic extracts. (11)

Materials and Methods

The Extraction:

Phenols were extracted from green tea by taking 40grams of powdered green tea by using the solvent methanol 9 to 1 water then make the extraction by 1 water to 1 methanol. in the two steps Leaves the extract to the second day after that filtered the extract and evaporate to a third by rotor evaporator and then separated with a separating funnel where added the chloroform to the mixture by (1:1) with mixing two layers will appears as (chloroform + water) take a layer of water and dry up we get After a drying with lyophilizer a dark powder, which is detected by the use of ferric chlorides concentration of 1% which

gives the green color or blue when mixed in solutions containing phenols(12).

Susceptibility test

The effect of drug cefixime have been studied in the growth of two types of bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, which were obtained from the Department of Biology in the College of Science / University of Mosul, and the antimicrobial activity of the drug was studied by using the disk diffusion method based on Bauer et.,al 1966, where cultivating the different kinds of microorganisms in the nutrient broth and incubated at 37°C for 24 hours. Then make a different concentrations from the materials under the study, namely, (600,400,200,100,50,25,12.5) mg / ml and added 1 ml from each concentration of these concentrations to tubes each containing 100 disk of a filter paper (Whatman No.1) in diameter of 6 mm after sterilized by the autoclave. Then inoculated a number of petri dishes containing nutrient agar media with 0.1 ml of the bacterial suspension and separated on the surface of the media then placed in each plate a disk for each concentration of the substance to be considered after that incubation the plates in 37°C for 24 hours, and measurement of the diameter of inhibition zone surrounding the disk.

Results and Discussion

Table (1):- The effect of phenolic extracts in the growth of *Escherichia coli* bacteria.

Percentage %	diameter of inhibition Mm	concentration Mg/ml
100a	14	600
85a	12	400
71 b	10	200
57 be	8	100
42 cd	6	50
0 de	0	25
0 e	0	12.5

Similar letters indicate no significant difference and different letters indicate significant differences by Duncan test at the level of probability 0.05 and calculated the percentage based on the highest concentration.. Table (1) the effect of phenolic extracts on the growth of bacteria *Escherichia coli* at 37c on nutrient agar media for 24 hours, phenolic extracts inhibit the growth of bacteria by 85% at the concentration 400 mg / ml diameter of 12 mm compared with the highest concentration and 71% inhibition with a diameter of 10 mm at the concentration 200 mg / ml. When the concentration 100 mg / ml inhibition zone of 8 mm in diameter. While the concentration 50 mg / ml the percentage of inhibition of 42%

of the highest concentration, the inhibitory effect of the phenolic extracts result from there effect on the hydroxyl group when produce hydrogen bound with the active site of the enzymes and inhibition the metabolic activity of the microorganisms⁽¹⁵⁾,and these results were in agreement with other studies Al- Anzi and Farag ^(14,15) that the phenolic extracts effected on the growth of microorganisms and there have been significant differences between treatments at the level of probability 0.05 According to Duncan test

Table 2: the impact of drug cefixime in the growth of bacteria, *Escherichia coli*

Percentage %	diameter of inhibition Mm	concentration Mg/ml
100 a	32	200
93 ab	30	100
87 bc	28	50
82 c	24	25
61 c	20	12.5

Similar letters indicate no significant difference and different letters indicate significant differences by Duncan test at the

level of probability 0.05 and calculated the percentage based on the highest concentration.. Table (2) the effect of drug

cefixime at the same experimental conditions as there were different effects in the growth of *Escherichia coli* at the concentration 200 mg / ml there was complete inhibition to the growth of bacteria 100% and the diameter of inhibition was 32mm at the concentration 100 mg / ml and the rate of inhibition was 88% from the highest concentration and the diameter of inhibition was 30mm either at the

concentration 50 and 25 mg / ml the rate of inhibition was 87% and 82% from the highest concentration and this is consistent with Oudhuis et.,al 1998 who mentioned that the drug cefixime was influential in gram positive and negative bacteria. There were significant differences among treatments according to Duncan test at the level of probability 0.50.

Table (3):- the impact of drug cefixime after adding phenolic extracts in the growth of bacteria, *Escherichia coli*

Percentage %	diameter of inhibition Mm	concentration Mg/ml
100 a	34	200
88 b	30	100
82 bc	28	50
76 cd	24	25
52 d	18	12.5

Similar letters indicate no significant difference and different letters indicate significant differences by Duncan test at the level of probability 0.05 and calculated the percentage based on the highest concentration.. Table 3 shows the effect of drug cefixime after adding phenolic extracts in the growth of bacteria *Escherichia coli*, as showed that there are different degrees of effect. At the concentration 200 mg / ml the diameter of inhibition was 34mm and the rate of inhibition from the highest concentration at the concentration 100 mg / ml 88% and the diameter is 30mm. while at the concentration 50 mg / ml the rate of inhibition was 82% with a diameter

28mm while at the concentration 25 mg / ml the rate of inhibition was 76%, diameter 24mm, while at the concentration 12.5 mg / ml the rate of inhibition was 52% and the diameter of inhibition was 18mm from this results we are concluded that the drug was less effective after the addition of the phenolic extracts and antagonism was occurred between them whichever the phenolic extracts had reduced the effect of the drug, which showed inhibitory effect on the growth of bacteria before the addition of the phenolic extracts.

Table (4):- study the effect of phenolic extracts in the growth of bacteria, *Pseudomonas aeruginosa*

Percentage %	diameter of inhibition Mm	concentration Mg/ml
100 a	16	600
87 a	14	400
75 b	12	200
62 bc	10	100
50 cd	8	50
0 de	0	25

0 e	0	12.5
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Similar letters indicate no significant difference and different letters indicate significant differences by Duncan test at the level of probability 0.05 and calculated the percentage based on the highest concentration.. Table (4) study the effect of phenolic extracts in the growth of bacteria, *Pseudomonas aeruginosa* at 37c on nutrient agar for 24 hours, the phenolic extracts inhibit the growth of bacteria (87%) at the concentration 400 mg / ml in diameter 14mm, compared with the highest concentration at 75% and the diameter of inhibition is 12mm at the concentration 200 mg / ml. While at the concentration 100 mg / ml the diameter of inhibition is 10mm. either the concentration 50

mg / ml the rate of inhibition was 50% from the highest concentration and these results were in agreement with other studies Al- Anzi and Farag^(14,15) that the phenolic extracts effect on the growth of microorganisms. the inhibitory effect of the phenolic extracts result from there effect on the hydroxyl group when produce hydrogen bound with the active site of the enzymes and inhibition the metabolic activity of the microorganisms⁽¹⁵⁾, and there have been significant differences between treatments at the level of probability 0.05 According to Duncan test.

Table (5):- Study the impact of drug cefixime in bacteria *Pseudomonas aeruginosa*

Percentage %	diameter of inhibition Mm	concentration Mg/ml
100 a	27	200
93 a	25	100
85 ab	23	50
78 ab	21	25
67 b	18	12.5

Similar letters indicate no significant difference and different letters indicate significant differences by Duncan test at the level of probability 0.05 and calculated the percentage based on the highest concentration.. Table (5) the effect of drug cefixime in bacteria *Pseudomonas aeruginosa* at the same experimental conditions as there were different effects in the growth of these bacteria. On concentration 200 mg / ml there was complete inhibition of the growth of bacteria at 100% and the diameter of inhibition was 27mm at

the concentration 100 mg / ml at 93% from the highest concentration with diameter of inhibition 25mm either at concentrations of 50 mg / ml and 25 mg /ml the inhibition was 85% and 78% from the highest concentration and there were significant differences among treatments according to Duncan test at the level of probability 0.50.

Table (6):- study the impact of drug cefixime after adding phenolic extracts in the growth of bacteria, *Pseudomonas aeruginosa*

Percentage %	diameter of inhibition Mm	concentration Mg\ml
100 a	25	200
92 a	23	100
84 ab	21	50
72 a	18	25
72 b	18	12.5

Similar letters indicate no significant difference and different letters indicate significant differences by Duncan test at the level of probability 0.05 and calculated the percentage based on the highest concentration.. Table (6) shows the effect of drug cefixime after adding phenolic extracts in the growth of bacteria, *Pseudomonas aeruginosa*, as showed that there are different degrees of effect. On the concentration 200 mg / ml the diameter of inhibition was 25mm and the percentage of inhibition of highest concentration on 100 mg / ml is 92 %, and 23mm in diameter and at the concentration of 50 mg / ml inhibition rate was 84% and 21mm in diameter at the concentration 25 and 12.5 mg / ml inhibition rate was 72%, diameter 18mm and there were differences among treatments according to Duncan test. Cefixime is an orally absorbed cephalosporin and which has a wide spectrum of antimicrobial activity against several gram-positive and gram-negative bacteria.^(17,18) the bactericidal effect of cefixime as a result of inhibits the third and final stage of bacterial cell wall synthesis by preferentially binding to specific penicillin-binding proteins (PBPs) that are located inside the bacterial cell wall. Penicillin-binding proteins are responsible for several steps in the synthesis of the cell wall and are found in quantities of several hundred to several thousand molecules per bacterial cell. Thus, the intrinsic activity of cefixime as well as the other cephalosporins and penicillins against a particular organism depends on its ability to gain access to and bind with the necessary PBP. Like all beta-lactam antibiotics, cefixime ability to interfere with PBP-mediated cell wall synthesis ultimately leads to cell lysis.⁽¹⁹⁾ In this research a study of the effect of phenolic compounds extracted from green tea interpenetrating with

drug cefixime against the bacteria *Pseudomonas aeruginosa*, *Escherichia coli* gives an idea of the overlap between each of them as the results showed that the drug cefixime gives high inhibitory effect in the bacterial species under study these results were in agreement with other studies^(20,21) which mentioned that the drug cefixime has a strong activity against gram-negative bacteria like *Pseudomonas aeruginosa*, *Escherichia coli* while this result disagreement with^(3,22,23) which find that the cefixime is inactive against *Pseudomonas aeruginosa*. before adding the phenolic extracts which was different influence in these types of bacteria but was less effective than drug cefixime which inhibits the growth of bacteria in lowest concentration This drug may be exert its effect by interfering with the structural cross linking of peptidoglycans in bacterial cell walls.⁽²³⁾ However, it is noted that the drug was less effective after the addition of phenolic extracts and antagonism was occurred between them whichever the phenolic extracts had reduced the effect of the drug, which showed inhibitory effect on the growth of bacteria before the addition of the phenolic extracts it was noted that it was less effective means that the combination led to a situation antagonism between each of the drug and phenolic extracts which can be interpreted as having occurred as a result of competition for effective sites in the bacterial cell wall and link phenolic extracts in those locations has reduced the association of the drug and the phenolic extracts was less influential, the different types of antibiotic have a certain mechanism of action in the microorganisms, whether on the cell wall or enzymes or nucleus and nucleic acids⁽⁹⁾ Therefore, the continuation of antagonism from high concentrations to the concentrations of the lowest among the phenolic

extracts and the drug makes of interpenetrate between the components of green tea negative interpenetrating which reduces the effect of the drug on the microorganisms.

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