Study of Synergistic Effect of Allicin with Antibacterials Against Micro-organisms

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Background: This study was designed to observe the antibacterial activity of allicin in combination with antibacterials, ciprofloxacin, enoxacin, vancomycin and clarithromycin against microorganisms pseudomonas aureginosa and staph aureus.

Study Design: It is an analytical study.

Materials and methods: Allicin was extracted from garlic with ethanol and chloroform. It was isolated by filteration technique using 15 mm Hg of pressure. Antibiotic discs were taken and micro-organisms were obtained from pathology lab FJMC LHR, Antibacterial activity of allicin was observed in combination with antibiotics.

Results: It was found from the study that there is strong synergistic effect of allicin in combination with ciprofloxacin and enoxacin against pseudomonas aureginosa. Allicin also showed synergistic antibacterial effect with vancomycin and clarithromycin against staph aureus.

Conclusion: Due to the increasing resistance developed by microorganisms against the antibiotics, it may be suggested that the use of allicin along with antibiotics may overcome this resistance.

Key words; Allicin, ciprofloxacin, enoxacin, vancomycin, clarithromycin, antibacterial activity.

Introduction

Antibacterial agent is one of the major advances of the medicine. Conventionally, antibacterial drugs have been developed on the basis of their ability to inhibit bacterial multiplication. Antimicrobial resistance has been an issue since the introduction into clinical use of the first agents in the 1940s.[1]

This decline in antibacterial drug discovery, coupled with increasing risk as a result of infections caused by drug-resistant bacterial pathogens, represents a clear public health threat.[2]

The use of botanicals and nutritional supplements has increased dramatically in the last two decades, with sales approaching five billion dollars (the use in 1998), Garlic has a wide spectrum of actions; not only is it antibacterial, antiviral, antifungal and antiprotozoal, but it also has beneficial effects on the cardiovascular and immune systems. Allicin, an active ingredient of garlic, possesses a range of antimicrobial, antifungal properties. It is found that plant enzyme allinase and its substrate alliin, generate allicin.[3]

Louis pasture was the first to describe the antibacterial effect of onion and garlic juice.

Historically, garlic has been used worldwide to fight bacterial infection. Allium vegetables, particularly garlic exhibits a broad antibiotic spectrum against both gram positive and gram negative bacteria.[4] Allicin in its pure form exhibits anti bacterial activities against a wide range of gram positive and gram negative bacteria; including multi drug resistant enterotoxicogenic strains of E-coli.[5] Allicin (thio-2-propene-1-sulfinic acid S-allyl ester) is the main biologically active component of garlic clove extracts. Its biological activity was attributed to either antioxidant activity or thiol disulfide exchange. This study demonstrates that in addition to its antioxidant activity, the major biological effect of allicin should be attributed to its rapid reaction with thiol containing proteins. Here, we focus primarily on an alternative novel strategy for antibacterial drug development that could potentially alleviate the current situation of drug resistance — targeting non-multiplying latent bacteria, which prolong the duration of antimicrobial chemotherapy and so, might increase the rate of development of resistance.[6]

Present study was designed to observe the antibacterial action of allicin alone and also in combination with other antibacterials like ciprofloxacin, enoxacin, vancomycin and clarithromycin against gram+ve and gram-ve microorganisms.

Materials and Methods

Antimicrobial discs and media were purchased from their respective manufacturers. Test material garlic was purchased from local market and its active compound allicin was isolated from PCSIR laboratories lahore. Micro-organisms psudomonas aureginosa and staph aureus were obtained from microbiology laboratory (FJMC Lahore). Allicin was dissolved in nutrient broth with dilutions of 1:50, 1:80 and 1:120.

Antibacterial tests were performed on Muller Hinton agar plates according to the procedure outlined by the NATIONAL Committee for Clinical Laboratory Standards[7]. Plates containing staph aureus and pseudomonas were incubated with antibiotic discs alone and along with allicin for 24 hrs at 37°c. A concentration of 1:500 of allicin was tested for

stasis to see no zone of inhibition. Results in this study were presented as Mean+SEM (standard error of mean) and also by regression analysis. P value with significant decimals is provided where ever needed.

Results

Sensitivity pattern (mm) synergism of Allicin (1:50 dilution) and Ciprofloxacin and its comparison with the sensitivity pattern (mm) of Ciprofloxacin (alone) against gram negative Pseudomonas aureginos.

Herb/drug	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard Div	P-value
Allicin plus Ciprofloxacin	02	29	19.6	8.9	N.S
Ciprofloxacin (alone)	00	28	18.1	9.1	

N.S= No significant difference

Sensitivity pattern (mm) synergism of Allicin (1:50 dilution) and Enoxacin and its comparison with the sensitivity pattern (mm) of Enoxacin (alone) against gram negative Pseudomonas aureginosa.

Herb/drug	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard Div	P-values
Allicin plus Enoxacin	02	28	14.4	5.2	N.S
Enoxacin (alone)	00	22	14.6	5.6	

N.S= No significant difference

Sensitivity pattern (mm) synergism of Allicin (1:50 dilution) and Vancomycin and its comparison with the sensitivity pattern (mm) of Vancomycin (alone) against gram positive Staph aureus.

Herb/drug	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard Div	P-value
Allicin plus Vancomycin	02	25	13.9	6.5	P<0.001
Vancomycin (alone)	00	16	12	3.7	

P<0.001= Highly significant difference

Sensitivity pattern (mm) synergism of Allicin (1:50 dilution) and Clarithromycin and its comparison with the sensitivity pattern (mm) of Clarithromycin (alone) against gram positive Staph aureus

Herb/drug	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard Div	P-value
Allicin plus Clarithromycin	00	19	9.8	7.7	P< 0.01
Clarithromycin (alone)	00	25	10.4	8	

P< 0.01= significant difference

Internationally Standardized Interpretations

S= Sensitive

I= Intermediate sensitive

R= Resistant

Drug	S	I	R
Enoxacin	≥ 18	15 -17	≤ 14
Ciprofloxacin	≥ 21	16 -20	≤ 15
Vancomycin	≥ 12	10 -11	≤ 9
Clarithromycin	≥ 18	14 -17	≤ 13

Discussion

The sensitivity pattern of allicin in combination with ciprofloxacin and its comparison with ciprofloxacin alone against-gram-negative Pseudomonas aureginosa was noted. In this mean zone of inhibition of allicin and ciprofloxacin was more as compared to the mean zone of inhibition of ciprofloxacin alone but this shows no significant difference. Correlation of coefficient was also calculated and graph of regression analysis showed a direct relationship between the sensitivity of ciprofloxacin and its synergistic effect with allicin. This shows a highly significant difference. (p<0.001)

In the present study, we also observed the sensitivity pattern of synergism of allicin and enoxacin and its com-

parison with the sensitivity pattern of enoxacin alone against gram negative pseudomonas aureginosa. In this study the mean zone of inhibition of allicin plus enoxacin was less as compared to mean zone of inhibition of enoxacin alone but this shows no significant difference. Graph of regression analysis showed a direct relationship between enoxacin and its synergistic effect with allicin. This is a highly significant difference ($p<0.001$).

It is known that ciprofloxacin and enoxacin inhibit DNA gyrase (enzyme) and interfere with DNA replication, repair and transcription and ultimately cause bacterial cell death. In high concentrations, they cause a dose-dependent inhibition of RNA synthesis which actually results in bacterial killing.[8.]

The main antimicrobial action of allicin is due to its chemical reaction with thiol groups of various enzymes e.g. alcohol dehydrogenase, thioredoxin reductase and RNA polymerase.[9] So the combination of allicin with antibacterial could inhibit both antibiotic-susceptible and antibiotic-resistant pathogenic bacteria and most combinations are synergistic or additive.

In our data, the sensitivity pattern of synergism of allicin and vancomycin and its comparison with the sensitivity pattern of vancomycin alone against gram positive staph aureus was also observed. In this step, the mean zone of inhibition of allicin plus vancomycin was more than the sensitivity pattern of vancomycin alone; and it shows highly significant difference ($P<0.001$). Vancomycin has been used over the past decade because of the increasing incidence of methicillin resistant staph. It has bactericidal action and decreased adverse reactions.[10]

The first report of staph aureus with resistance to vancomycin was from japan in 1997 by Hiramatsu et al raising the threat of incurable staph infections. As powerful antibiotics lose their punch against "super bugs" such as vancomycin resistant enterococci and methicillin resistant staph aureus, scientists are searching for new antimicrobial agents from natural sources. They found that allicin, the major component of garlic, is one such agent and it was recently shown to be potent against VRE (vancomycin resistant enterococci) and methicillin resistant staph aureus.[11]

Lastly the synergistic effect of allicin and clarithromycin and its comparison with clarithromycin alone was observed against gram-positive staph aureus. The mean zone of inhibition of allicin + clarithromycin was less as compared to the mean zone of inhibition of clarithromycin alone but it shows a significant difference ($P<0.01$). There is no documented study about the synergistic effect of allicin and clarithromycin.

Clarithromycin inhibit bacterial protein synthesis by binding reversibly to the subunit 50 s of bacterial ribosome, thereby inhibiting translocation of peptidyl-t RNA. So action is bacteriostatic[12] as the action of allicin is also bacreriostatic so the combination of two bacteriostatic agents is bactericidal.

Conclusion

Antibacterial activity of allicin alone was less than ciprofloxacin and enoxacin against pseudomonas aureginosa. This may explain that the antibacterials which are recently introduced in pharmaceutics have potent bactericidal effects as compared to allicin.

Allicin also showed synergistic antibacterial effect with vancomycin and clarithromycin against staph aureus.

So it is found that allicin has a synergistic effect when it combines with antibiotics.

Finally we may conclude that due to increasing resistance of current and old antibiotics, it may be suggested that the use of allicin along with antibiotics may overcome the resistance developed by microorganisms. However further research is needed in-vivo as well as in-vitro to reach a better conclusion.

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