ORIGINAL ARTICLE

The combination effect of curcumin with different antibiotics against *Staphylococcus aureus*

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The different investigation has been carried out on the biological activities of curcumin but the effect of this natural product on the antibacterial activity of different antibiotics has not been demonstrated. In this study the enhancement effect of curcumin on the antibacterial activity of different antibiotics was evaluated against *Staphylococcus aureus*. Disk diffusion method was used to determine the antibacterial activity of these antibiotics in the absence and presence of sub inhibitory concentration of curcumin. A clinical isolate of *S. aureus* was used as test strain. In the presence of sub-inhibitory concentration of curcumin (500 µg/disc) the antibacterial activities of cefixime, cephotaxime, vancomycin and tetracycline have been increased against test strain. The highest fold increase in area was observed for cefixime against *S. aureus* (a 52.6 % increase in inhibition zone surface area). Also the increases in inhibition zone areas (%) for cephotaxime, vancomycin and tetracycline were 24.9%, 26.5% and 24.4%, respectively. No enhancing effect on the antibacterial activities of others antibiotics was detected against *S. aureus* at content of 500µg/disc. Conversely, in case of nalidixic acid, curcumin showed an antagonistic effect on the antibacterial activity of this antibiotic against test strain. These results signify that the curcumin potentiates the antimicrobial action of cefixime, cephotaxime, vancomycin and tetracycline suggesting a possible utilization of this edible compound in combination therapy against *S. aureus*.

Key words: Antibacterial activity, combination effect, *Staphylococcus aureus*, curcumin

INTRODUCTION

Curcumin [diferuloylmethane; (1E, 6E)-1,7-bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione] known as Indian solid gold is the small-molecular-weight, major wide spectrum biological active, non-volatile pigmentary polyphenolic component of curry spice turmeric (*Curcuma longa*). Turmeric widely used as a spice, food preservative and coloring material in East. It has been thousands of years that this golden colored spice is used in Indian system of holistic medicine, known as Ayurveda, and other traditional medicine specially as a treatment for various respiratory condition and also other diseases like liver disorders, anorexia, parasitic infections, rheumatism, diabetic wounds, sinusitis, cold and flu symptoms.[1]

Modern world researchers demonstrated many different valuable pharmacological effects for this natural product. In gastrointestinal system it is gastro protective and anti ulcer in stomach, antispasmodic and antiflatulent in intestine, hepatoprotective and improve pancreas tasks. In cardiovascular system it is a cardioprotective and has hypocholesteremic effect. Also it improves the lipid profile and have neuroprotective, anti-inflammatory, antioxidant, anticarcinogenic by inducing apoptosis, Pro/antimutagenic, anticoagulant, antifertility, antidiabetic, antiviral, antifibrotic and antiprotozoal activities.[2,3] Moreover, curcumin [Figure 1] interacts with numerous molecular targets in body. It strongly inhibits the activation of different transcription factors that regulate the expression of genes that contribute to tumorgenesis, cell survival, inflammation, invasion, cell proliferation and angiogenesis.[1,4] Also the antimicrobial activity of turmeric as well as its main constituent (curcumin), and its different derivatives such as curcuma oil, has been published in literature.[5-8]

The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems, leading to treatment drawbacks for a large number of drugs.[9,10] Consequently there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy.[11-13] Recently some edible natural products and food ingredients have been reported to enhance the antibacterial activity of different antibiotics such as nitrofurantoin, clindamycin.[14-16] As mentioned above the different investigation has been carried out on the biological activities of curcumin but the combination effects of this natural product with different antibiotics have not been demonstrated. In this study we investigated the effect of curcumin on the antibacterial activity of different antibiotics against *Staphylococcus aureus*. Curcumin has been reported having an extremely good safety profile and no toxicity observed when taken at doses as high as 12 g/day.[1,17]

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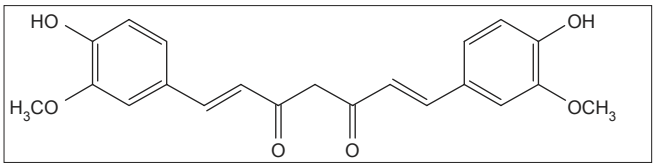

Figure 1: The structure of curcumin

Taken together, this compound as a highly safe compound may be considered for combination therapy against *S. aureus*, due its potential synergistic effect with important antibiotics such as cefixime, cephotaxime, vancomycin and tetracycline.

MATERIALS AND METHODS

Curcumin Preparation

Curcumin 65-70% (Product code # C 1386) was purchased from Sigma. The Curcumin 65-70% was further purified according to the previous work[18] using silica column chromatography and dichloromethane as solvent. The isolated curcumin was also crystallized with methanol-water.[1,9] as orange needle crystals. Physicochemical characteristics of the obtained curcumin were in agreement with literature.[18] TLC experiments showed that the curcumin was completely free of demethoxycurcumin and bisdemethoxycurcumin.

Disk Diffusion Assay to Evaluate Combined Effects

A disk diffusion method was used to assay the 24 different antibiotics for bactericidal activity against test strains on Müeller-Hinton agar (Merck, Germany) plates. The standard antibiotics disks were supplied from Mast Co., UK. To determine combined effects, each standard paper disc was further impregnated with sub-inhibitory concentration of curcumin (500 µg). A single colony of test strains were grown overnight in Müeller -Hinton broth medium on a rotary shaker (200 rpm) at 35°C. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard and prepared disks containing of curcumin (500µg/disc). Clinical isolates of *S. aureus* from our culture collection were used as test strains. Similar experiments were carried out with curcumin alone. After incubation at 37°C for 24 hrs, the zones of inhibition were measured. The assays were performed in triplicate.

RESULTS AND DISCUSSION

Therapeutic roles for curcumin in different diseases have been established in recent years .Curcumin has a very good potential to move into the clinic and may proved to be "Curecumin".[1] In this investigation the effect of curcumin on the antibacterial of different antibiotics was investigated against *S. aureus* using disk diffusion method. The diameter of inhibition zones (mm) around the different antibiotic disks with or without of curcumin against test strains are shown in [Table 1]. The antibacterial activities of cefixime, cephotaxime, vancomycin and tetracycline have been increased in the presence of curcumin against test strain. The highest fold increases in area were observed for cefixime against *S. aureus* (a 52.6%-fold increase). The increases in inhibition zone areas (%) for cephotaxime, vancomycin and tetracycline were 24.9%, 26.5% and 24.4%, respectively. No enhancing effect on the antibacterial activities of others antibiotics was observed against *S. aureus* at tested concentration. Conversely, for nalidixic acid, curcumin showed an antagonistic effect on the antibacterial activity of this antibiotic against the test strain. It should be pointed out that the curcumin content of 500 µg/disc was chosen to guarantee that the effect produced was due to the combination and not to the effect of the curcumin itself. So the effect observed in this condition could be due to the antibiotic-curcumin combination. At the concentration tested, curcumin significantly improved antibiotic efficacy against *S. aureus* when combined with cefixime, cephotaxime, vancomycin and tetracycline [Table 1].

At this time the reason of these enchantments and the reason for theses differences are not known and merits investigation. Efflux transporter mediated bacterial resistance to different antibiotics[19] and curcumin may inhibit this efflux pump system. This is the first report of combination effect of curcumin derived from *Curcuma longa* with different antibiotics. Today, curcumin as a food ingredient has drawn the attention of scientists because of its extensive pharmaceutical properties. In different phase I clinical trials, no toxicity except mild nausea and diarrhea was observed when taken at doses as high as 12 g/day and it is reported as an attractive choice for many disease therapies.[1,17]

CONCLUSION

Recently some edible natural products and food ingredients have been evaluated for increasing the antibacterial activities of different antibiotic .We showed that cinnamon essential oil and its major component (Trans-cinnamaldehyde) enhanced the antibacterial activity of clindamycin against a toxicogenic strain of *Clostridium difficile*. Also the enhancement activity of different essential oils (*Mentha longifolia* L. and *Mentha spicata* L.) and different monoterpenes (piperitone, carvone and menthone) on the antibacterial activity of nitrofurantoin has been previously reported.[14-16] In this study using disk diffusion assay we showed that the antibacterial activity of cefixime, cephotaxime, vancomycin and tetracycline can be increased by curcumin. A clinical isolates of *S. aureus* was select as test strain. *S. aureus* is common cause of different infection disease.[20] Today, *S. aureus* has become

Table 1: Zone of inhibition (mm) of different antibiotics against test strains (in absence and in presence of curcumin at content of 500 µg/disk)

Antibiotics (µg/disk)	*Staphylococcus aureus*		Increase in fold area (%) [a,b]
	Antibiotic only (A)	Antibiotic plus curcumin[b] (B)	
Cefixime (5)	17	21	52.6
Ciprofloxacin (5)	26	26	0
Amikacin (30)	30	30	0
Vancomycin (30)	17	19	24.9
Ceftazidime (30)	12	12	0
Penicillin G 10 UT	28	28	0
Cefepime (30)	24	24	0
Cephalexine 30	29	29	0
Nitrofurantoin 300	25	25	0
Tobramycin 10	30	30	0
Bacitracin 0.04	0	0	0
Tetracycline (30)	24	27	26.5
Carbenicillin 100	28	28	0
Rifampicin 5	35	35	0
Clindamycin 2	24	24	0
Cephotaxime (30)	26	29	24.4
Erythromycin 5	10	10	0
Gentamicin 10	26	26	0
Streptomycin 10	25	25	0
Kanamycin 30	29	29	0
Ticaracillin 75	22	22	0
Co-trimoxazole 25	30	30	0
Methicillin 5	22	22	0
Nalidixic acid 30	12	0	Antagonist

[a]Mean surface area of the inhibition zone (mm^2) was calculated for each tested antibiotic from the mean diameter. The percent of Increases in inhibition zone area for different antibiotics against *Staphylococcus aureus* were calculated as $(b^2-a^2)/a^2 \times 100$ where *a* and *b* are the inhibition zones A and B, respectively. [b]All experiments were done triplicate and standard deviations were negligible.

resistant to many commonly used antibiotics.[21] The result demonstrated that curcumin as a safe natural product could also serve as valuable probes to study the structure-function relationships of the antibiotic resistance reversal agents. Therefore, this compound or its future derivatives have a good potential for combination therapy against *S. aureus*.

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