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Full Length Research Paper

In vitro evaluation of the interaction between tea extracts and penicillin G against *staphylococcus aureus*

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The herb-drug interaction between tea (*Carmelia sinensis*) extract and penicillin G (Pen G) was investigated against three strains of *Staphylococcus aureus* using pair combinations in an *in vitro* decimal assay for additivity test. Results showed that the interactions between penicillin G and tea extracts were mainly additive against the three strains of *S. aureus.* This suggests that the concomitant administration of tea and Pen G may not impair the antimicrobial activity of Pen G.

Key words: Penicillin G, tea extract, herb-drug interaction.

INTRODUCTION

The need to combat microbial resistance to antibiotics is an increasing global concern (Kunin, 1993; Twomey, 2002). *Staphylococcus aureus* is one of the gram-positive microorganisms that have been shown to exhibit resistance to a wide range of commonly available antibiotics, especially the penicillins (Ghobashy et al., 1994; Haldane and Affias, 1981). Therefore, penicillins are often administered in combination with other antibiotics in the treatment of resistant (or suspected resistant) bacterial infections (Wise et al., 1969; Okore, 1990). Combined antibiotic therapy has been shown to delay the emergency of bacteria resistance and may also produce desirable synergistic effects in the treatment of bacteria infection (Elopoulos, 1982).

In Nigeria, antibiotics are sometimes willfully or inadvertently administered concomitantly with herbs or beverages (Nwafor et al., 2003; Esimone et al., 2003a,b). This portends a potential herb-drug interaction, which could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome). One of the herbs that are widely consumed concomitantly with most drugs is tea. Tea is one of the most widely drunk beverages in the world today. (Hamilton-Miller, 1995). Recent works on tea have shown that it has some medicinal properties including antimicrobial effect against a wide range of bacteria, fungi and viruses (Sakanata et al., 1989; Toda et al., 1991).

In this report, we attempted to evaluate the antimicrobial interaction between tea extracts and penicillin G against three strains of *S. aureus in vitro* using the decimal assay for additivity (DAA) method previously described (Sanders et al., 1993). The DAA method has been shown to eliminate some of the shortcomings of other methods of antibiotic evaluation. Also the use of DAA helps to identify the drug ratio at which the interaction in maximal (Sanders et al., 1993). Sanders et al. (1993) suggested that comparing the amount of each drug required to produce the interaction could help to predict whether the interaction may occur at

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Table 1. Susceptibility of *S. aureus* strains to tea and penicillin G.

DRUG	Strain I (From burn Patient)	Strain II (From burn Patient)	Typed Culture ATCC 12600
Tea	10 mm	4 mm	8 mm
Penicillin G	16 mm	12 mm	18 mm

clinically attainable concentration.

MATERIALS AND METHODS

Culture media

Agar (oxoid) and nutrient broth (Merck) were used, and they were prepared according to the manufacturer's specifications.

Test micro-organisms

Three different strains of *Staphylococcus aureus* were used. Two of these were hospital isolates from burn patients and the third was a typed isolate (ATCC 12600). The two isolates from burn patients were purified using standard techniques.

Drugs

The drugs used for this work were a commercial tea sample (NDU TEA®, Cameroon) and the pottasium salt of benzypenicillin B.P.® (Shanghai Pharm and Chem. Co. Ltd., China).

Extraction of tea sample

Ten grams of the commercial NDU tea sample were weighed and boiled on 50 ml of water for 5 min. The suspension was filtered and evaporated to dryness in an evaporating dish over a hot water bath. The required amount of tea extract was weighed out and dissolved in the required amount of water to obtain the desired concentration of tea extract solution.

Preparation of Pen G and tea discs

Circular filter paper discs (6 mm diameter) were prepared with the aid of an office paper perforator. The discs were placed in a Petri dish and sterilized in an autoclave. Dilutions of several concentrations of the tea and penicillin G were made in test-tube using sterile water. The paper discs were then aseptically transferred into the tubes containing the drugs solutions and allowed to absorb the solutions for about 15 s. The discs were then aseptically transferred to empty sterile test tubes and allowed to air-dry while the mouth of the test tubes is still plugged with cotton wool. Each of the test tubes containing the dried discs was labeled with the strength of the solution of drug in which the paper discs were dipped. We had previously established that the 6 mm disc has a capacity of absorbing 10 µl of drug solution at saturation.

Susceptibility testing

Exactly 20 ml of molten nutrient agar in sterile Petri dish was seeded with 40 µl of the standardized test microorganism, well distributed and allowed to set on a horizontal plane. Discs containing Pen G or tea extracts soaked in solution of 50 mg/ml (equivalent to 500 µg/disc) were aseptically placed on the seeded nutrient agar plates. This procedure was performed in 4 replicate plates for each test microorganism and for both drugs. After a 30 min pre-diffusion time interval, the plates were incubated at 37°C for 24 h. Thereafter, the diameters of zones of inhibition surrounding the discs were accurately measured and their relative susceptibility pattern deduced.

Standard dose response curves of tea and penicillin G

Several sterile Petri dishes were seeded with 40 µl of standard test microorganism and allowed to set. The prepared discs containing different drug masses were placed on the dishes. This was performed in four replicate plates for each microorganism. After a 30 min pre-diffusion time interval, the plates were incubated at 37°C for 24 h after which time the inhibition zone diameter (IZD) surrounding the discs were measured. The definite drug masses and their corresponding IZDs were used to construct the standard dose-response curve for penicillin G and tea. From this curve, a target IZD was selected from their mid-range so that increases or decreases on IZD size due to any interaction could be detected. The corresponding drug mass of the target IZD was obtained by linear regression analysis.

Determination of the combined activity of tea extract and Penicillin G

The corresponding drug masses of both tea and penicillin G obtained from the procedures above was then used to prepare stock solutions of both drugs. These stocks were then mixed in such a way that each mixture contains from zero parts of penicillin G and ten parts of tea to ten parts of penicillin G and zero parts of tea. The discs were then prepared as described above and then placed on the seeded agar plates. Triplicates of this process were reproduced for each of the strains of *S. aureus* used for this work. After a 30 min pre-diffusion time interval, the plates were incubated at 37°C for 24 h, after which the IZD surrounding the discs were measured.

Data obtained were analysed by determining the confidence interval to ascertain if the differences in the mean diameters of the zones of inhibition were statistically significant.

RESULT AND DISCUSSION

The three strains of the test organism (*S. aureus*) were more susceptible towards penicillin G than the tea extract (Table 1). Also, the inhibition of the microorganisms by Pen G increased with increasing concentration of Pen G (Figure 1). On the other hand, increasing concentration of the tea extracts resulted in decreasing activity (Figure 1).

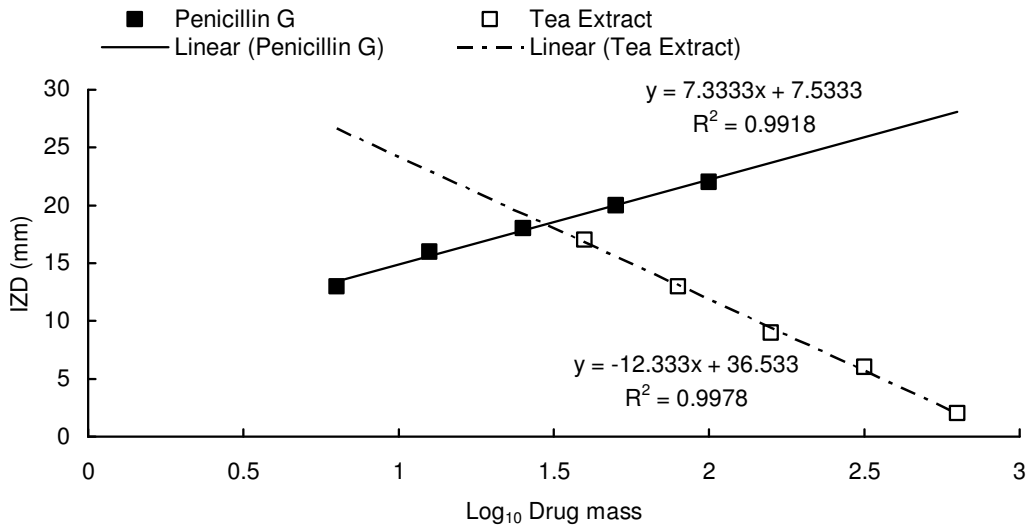

Figure 1. Standard dose-response curve of penicillin G and tea extract against strains of *S. aureus*.

Table 2. Result of the herb-drug interaction between tea and Pen G against the typed *S. aureus* ATCC 12600 evaluated by the DAA method.

Drug Combination	Range of IZD (mm)	Standard Deviation	Type of Interaction
10:0	41.71 – 39.59	0.82	
0:10	30.63 – 27.97	1.08	
1:9	41.15 – 31.83	3.77	Additivity
2:8	39.70 – 33.32	2.59	“
3:7	37.87 – 35.16	1.10	“
4:6	37.53 – 35.49	0.83	“
5:5	37.05 – 35.97	0.44	“
6:4	38.70 – 34.32	1.78	“
7:3	39.02 – 34.00	2.04	“
8:2	39.30 – 33.72	2.27	“
9:1	39.79 – 33.23	2.67	“

This could be attributed to the inability of higher concentrations of the tea extract to diffuse through the nutrient agar medium. This impairment in drug diffusion is a major limitation in the evaluation of the antimicrobial effects of plant extracts using the agar diffusion method. Usually, this problem is circumvented by allowing appropriate time for pre-diffusion. Although we allowed 30 min pre-diffusion in the present experiment, this appeared not to have significantly solved the problem of diffusion with the tea extract.

The result of the combination studies were additive (Tables 2 - 4) which shows that the inhibitory action of the combined agents were equivalent to the sums of the actions of the single agents (Sanders et al., 1993). Since both agents (tea and Pen G) inhibit bacteria by different mechanisms (Esimone, 2002b; Sabbath, 1982), an additive or synergistic interaction is expected to occur (Jawetz et al., 1952). Tea contains various tannins (polyphenols) such as epicatechin, epigallocatechin, which have been shown to exert profound antibacterial effects against a broad spectrum of bacteria, including *S. aureus* via membrane perturbations (Esimone et al., 2002b).

Perturbation of the cell membrane by tea results in a loss to free passage of materials in and out of cell leading to lysis of the cell, which eventually results in death. Pencillin G on the other hand, inhibits the third and final stage involved in the synthesis of peptidoglycan, which is a heteropolymeric component of the cell wall, which provides a rigid mechanical stability by virtue of its highly cross-linked lattice work structure. This cross linking is accomplished by a transpeptidation reaction that occurs outside the cell membrane (Sabbath et al., 1982). This double attack of both agents on different target sites of the bacteria could theoretically lead to either an additive or a synergistic effect (Jawetz et al., 1952). However,

Table 3. Result of the herb-drug interaction between tea and PenG against *S. aureus* strain II (from burn patient) evaluated by the DAA method.

Drug Combination of Penicillin G and Tea	Standard Deviation	Range of IZD (mm)	Type of Interaction
10:0	0.41	19.08 – 18.80	
0:10	0.41	7.20 – 6.20	
1:9	3.39	19.93 – 11.59	Additivity
2:8	1.96	18.17 – 13.35	“
3:7	2.05	18.28 – 13.24	“
4:6	0.42	16.28 – 16.24	“
5:5	0.21	16.01 – 15.51	“
6:4	0.89	16.85 – 1467	“
7:3	1.34	17.41 – 14.11	“
8:2	2.40	18.71 – 12.81	“
9:1	2.40	18.71 – 12.81	“

Table 4. Result of the herb-drug interaction between tea and PenG against *S. aureus* strain I (from burn patient) evaluated by the DAA method.

Drug Combination of Penicillin G and Tea	Standard Deviation	Range of IZD (mm)	Type of Interaction
10:0	0.82	32.31 – 30.29	
0:10	0.41	17.8 – 16.8	
1:9	2.76	32.29 – 25.51	Additivity
2:8	1.05	30.19 – 27.61	“
3:7	0.95	30.07 – 27.73	“
4:6	1.19	30.36 – 27.44	“
5:5	0.90	30.10 – 27.79	“
6:4	1.05	30.19 – 27.61	“
7:3	1.19	30.36 – 27.44	“
8:2	0.95	30.07 – 27.73	“
9:1	1.31	30.51 – 27.29	“

because the strains of *S. aureus* used were not resistant to any of the drugs, synergism is less likely to occur. Usually, the combination of two agents exhibit significant potentiation (synergism) only if the test organism is resistant to at least one of the agents.

At present, most *Staphylococci* isolated from individuals outside the hospital are resistant to penicillin G (Sabbath et al., 1982; Esimone and Adikwu, 2002) due to β-lactamases, which inactivate the drug. It is possible that tea could reverse such resistance and thus potentiate the effect of common penicillins against resistant *S. aureus*.

In conclusion, tea extracts did not impair the antimicrobial properties of penicillin G; rather, it enhanced its activity in an additive manner. Since several studies have shown that purified catechin fractions from green and black tea inhibit the growth of many bacteria species, concormitant administration of penicillins G with a cup of tea could be beneficial in the treatment of staphylococcal infections.

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