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In vitro Studies on the Interaction of Tea with Antimicrobial Agents

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Abstract: The *in vitro* interaction between a commercial variety of tea [*Camellia sinensis* L (Ternstroemiaceae)] and some antibiotics was evaluated against *Staphyloccocus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Synergism against *S. aureus* was exhibited by cloxacillin and tetracycline, while the effect of gentamycin was indifferent. Additive effect against *S. aureus* was exhibited by cefotaxime. The effects of streptomycin, ceftriaxone, ceftazidime, ciprofloxacin, ofloxacin and norfloxacin against *S. aureus* were antagonized. Gentamycin, tetracycline, cefotaxime and ceftazidime exhibited additive effects against *E. coli*. The activities of streptomycin, ceftriazone, ciprofloxacin, ofloxacin and norfloxacin against *E. coli* were antagonized. The results suggest a physicochemical basis for the interaction and indicate a potential for clinically relevant interaction when tea is consumed with antimicrobial agents.

Key words: Tea, *Camellia sinensis,* antimicrobial agents, interactions

INTRODUCTION

Tea consists of the prepared leaves of *Camellia sinensis* L (Hamilton, 1995) a shrub belonging to the Ternstroemiaceae and cultivated in India, Sri Lanka, China and Japan (Evans, 1989). The consumption of tea as a beverage (an infusion of the processed leaves) is worldwide. It is ranked as the most widely drunk beverage in the world (Stagg, 1980).

Literature is replete with documented research on tea. Some of the reported pharmacological activities include anti-inflammatory (Stagg, 1975) antioxidant and angiotensin inhibitory (Hattori *et al*., 1990) anticarcinogenic (Hattori *et al*., 1990; Zongmao, 1991) antihypertensive (Zongmao, 1991) antibacterial and antiviral (Toda *et al*., 1989) properties. Green tea has also been shown to inhibit lipid peroxidation in the kidneys, liver and testes of pre-treated animals as well as superoxide dismutase and catalase activities (Soussi *et al*., 2006) and prevent *Helicobacter pylori*-induced gastric epithelium damage possibly by inhibiting VacA (Ruggiero *et al*., 2007). It has also been credited with antidepressant effect (Singal *et al*., 2006) and shown to inhibit pancreatic phospholipase A2 and intestinal absorption of lipids in ovariectomized rats (Wang *et al*., 2006). Studies on antibacterial activity showed that extract of the leaves inhibited the growth of several micro organisms including *Staph aureus* and *E. coli* which were highly sensitive (Bandyopadhyay *et al*., 2005). In addition to antibacterial activity, extract of green tea has also been shown to possess antimycotic activity (Turchetti *et al*., 2005). Pharmacological activities are not restricted to the leaves as extracts of the flower have been shown to exhibit antiallergic properties by inhibiting the release of beta-hexosaminidase from cultured cells (Yashikawa *et al*., 2007) and to suppress serum triglyceride elevation in olive-oil treated mice (Yashikawa *et al*., 2005).

Phytochemical studies have shown that tea contains a complex mixture of constituents. The isolation of acylated oleanane-type triterpene oligoglycosides, floratheasaponins D-I, floratheasaponins A-C and floratheasaponins A-F (Yashikawa *et al*., 2007) as well as several flavonol glycosides and catechins (Yashikawa *et al*., 2007) from the tea plant have been reported. Among the elements reported in tea are notably manganese, fluorine, aluminum and selenium (Zongmao and Yongming, 1994). Other constituents include ascorbic acid, amino acids (Zongmao and Yongming, 1994) tannins (Hawley, 1981) and small quantities of theobromine, theophylline, volatile oil (Evans, 1989) caffeine, flavonols such as quercitin, kaempferol and myricetin and polyphenols like catechins (Kirk and Othmer, 1980; Hara and Watanabe, 1989).

An estimated 2.5 million cups of tea are consumed daily worldwide (Kirk and Othmer, 1980). This consumption pattern, coupled with its use sometimes as

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fluid for ingestion of orally administered drugs as well as its complex mixture of constituents, suggest a likelihood for interaction of tea with commonly used drugs such as antibiotics. Antibiotics are widely used and usually taken on prescription and self medication. In UK alone, antibiotic prescription is estimated to make up about one in every 6 prescriptions (Henry, 1991). Antibiotics prescription also accounts for 17-20% of drugs prescribed in Australia (Harvey, 1988). In Nigeria, it is a common practice for uninformed persons to take antibiotics even for prophylaxis at the slightest exposure to or suspicion of infection. They are generally freely available from many sources including itinerant hawkers in streets and open markets (Adenika, 1998). A recent study has also shown that more than 56% of outpatients in southeastern Nigeria obtain antibiotics without prescription.

This study was undertaken to investigate the effect of tea on the antimicrobial activity of selected antibiotics *in vitro*.

MATERIALS AND METHODS

Tea material: A commercial tea variety, Lipton® (yellow label; manufactured by Lever Brothers Nigeria PLC) purchased from a Supermarket Market in Nsukka, Nigeria, was used for the study. The tea bags were opened and 20 g of the tea was extracted by boiling in 100 mL of distilled water for about 45 min. Solvent removal afforded 30 mg of a semi-solid extract. Concentrations of 1.5, 2.0, 2.5, 3.0 and 3.5 mg mL^{-1} were obtained by diluting the extract with distilled water.

Test antibiotics: Antibiotic discs containing ampicillin (10 μg), cloxacillin (5 μg), gentamycin (10 μg), streptomycin (10 μg), tetracycline (25 mg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ciprofloxacin (10 μg), ofloxacin (10 μg) and norfloxacin (10 μg), were obtained from Jireh Laboratories, Nigeria and stored at 2-8°C.

Test microorganisms: *Staphylococcus aureus* (*S.aureus*) ATCC 12600 and *Escherichia coli* (*E. coli*) ATCC 11775 were obtained from the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics, University of Nigeria, Nsukka. The cultures were maintained on nutrient agar slants at 4°C. Subculturing of the microorganisms after incubation a 37°C for 18-24 h on nutrient agar was carried out before each experiment. Microbial suspension of about 1×10^9 CFU mL^{-1} were serially diluted to 1×10^7 CFU mL^{-1} for each organism and used.

Microbial susceptibility test: The susceptibility of the microorganisms to tea extract was evaluated using the agar well diffusion method (Lovian, 1980). Two drops (40 μL) each of 3.5, 3.0, 2.5, 2.0 and 1.5 mg mL^{-1} dilution of tea extract were put in appropriately labeled wells (8 mm diameter) made by means of a cork borer on gelled agar containing 1×10^7 CFU mL^{-1} of the test organism. Each plate contained five wells. The plates were incubated at 37°C for 24 h after which the Inhibition Zone Diameters (IZD) were measured with a rule.

Determination of interaction between tea extract and antibiotics: The interaction of the tea extract with antibiotics was evaluated using the overlay innoculum susceptibility disc method (Chinwuba *et al*., 1991). A sub-bacteriostatic concentration of the tea extract (1.5 mg mL^{-1}) in nutrient agar was used as the Test Agar Plate (TAP) while plain nutrient agar without the tea extract was used as the Control Agar Plate (CAP). Briefly, extract-agar mixture (20 mL) was placed in a petri dish to form the base-extract agar layer. Molten agar (5 mL) containing 1×10^7 CFU mL^{-1} of either *S.aureus* or *E.coli* was applied as a thin overlay innoculum agar layer and allowed to solidify. Antibiotic discs were placed on the solidified surface. The control contained an extract-free base agar layer. The plates were incubated at 37°C for 18-24 h. The clear Inhibition Zone Diameter (IZD) was measured using a rule. Three determinations were made for the test and control. Interaction was defined in terms of synergism (IZD increment of 19% or more), additivity (less than 19% increase in IZD), indifference (cases showing no variations in IZD) (Chinwuba *et al*., 1991) and antagonism (cases showing IZD of control> IZD of test).

Statistical analysis: Data obtained was analyzed using Student's t test and the results expressed as Mean±SEM.

RESULTS AND DISCUSSION

Results of susceptibility test showed that concentrations of tea extract up to 1.5 mg mL^{-1} were not inhibitory to the test cultures of *S. aureus* and *E. coli*. Thus 1.5 mg mL^{-1} was selected as a sub-bacteriostatic concentration of the tea extract and used in the interaction studies. Results of the interaction studies indicated varied degrees of activity against the tested organisms. Cloxacillin in combination with tea was synergistic against *S. aureus* while ampicillin in the presence of tea lost its activity against this organism (Table 1). Both cloxacillin and ampicillin were inactive against *E. coli* (Table 2)

Table 1: Interaction between tea and antibiotics against *S. aureus*

	Inhibition zone diameter (mm)			
Test antibiotic	Tea+antibiotic	Control	% Change	Inference
Ampicillin	NI	12.7±0.67	100.00	Antagonism
Cloxacillin	17.3±4.67	11.3±0.67	53.09	Synergism
Gentamycin	13.0±2.00	13.3±0.67	2.26	Indifference
Streptomycin	14.7±4.67	15.3±3.39	3.92	Antagonism
Tetracycline	18.3±2.67	14.7±8.19	24.49	Synergism
Ceftriazone	23.3±0.67	24.0±2.00	2.92	Antagonism
Cefotaxime	28.3±2.67	26.7±0.69	6.00	Additivity
Ceftazidine	19.7±0.67	20.0±2.00	1.50	Antagonism
Ciprofloxacin	19.3±0.67	22.0±0.00	12.27	Antagonism
Ofloxacin	18.3±0.67	19.3±0.59	5.18	Antagonism
Norfloxacin	12.3±.0.67	15.3±0.59	19.60	Antagonism

Values of Inhibition Zone Diameter (IZD) shown are Mean±SEM of 3 determinations (Student t test); NI = No Inhibition; Synergism = IZD increment of 19% or more; Additivity = less than 19% increase in IZD; Indifference = cases showing no variations in IZD; Antagonism = cases showing IZD of control >IZD of test

Table 2: Interaction between tea and antibiotics against *E.coli*

	Inhibition zone diameter (mm)			
Test antibiotic	Tea+antibiotic	Control	% Change	Inference
Ampicillin	NI	NI	0.00	-
Cloxacillin	NI	NI	0.00	-
Gentamycin	15.30±4.67	14.67±2.67	4.29	Additivity
Streptomycin	14.30±0.67	15.00±0.00	4.67	Antagonism
Tetracycline	18.67±0.67	16.30±0.67	14.54	Additivity
Ceftriazone	23.67±0.67	24.30±0.67	2.59	Antagonism
Cefotaxime	30.30±0.67	29.67±0.67	2.12	Additivity
Ceftazidine	19.67±0.67	18.67±0.67	5.36	Additivity
Ciprofloxacin	18.67±0.67	20.67±0.67	9.68	Antagonism
Ofloxacin	18.00±0.00	20.00±0.00	10.00	Antagonism
Norfloxacin	11.00±2.00	13.00±0.67	15.38	Antagonism

Values of Inhibition Zone Diameter (IZD) shown are Mean±SEM of 3 determinations (Student t test); NI = No Inhibition; Synergism = IZD increment of 19% or more; Additivity = less than 19% increase in IZD; Indifference = cases showing no variations in IZD; Antagonism = cases showing IZD of control >IZD of test

probably due to resistance by the strain of organism used. The activities of the fluoroquinolones-ciprofloxacin, ofloxacin and norfloxacin against *S. aureus* and *E. coli* were antagonized in the presence of tea (Table 1 and 2). For the aminoglycosides, gentamicin in combination with tea exhibited indifference against *S. aureus* (Table 1) and additive effect against *E. coli* (Table 2). The activities of streptomycin against *S. aureus* and *E. coli* were antagonized in the presence of tea (Table 1 and 2). Tetracycline exhibited synergism against *S. aureus* (Table 1) and additive effect against *E. coli* (Table 2). Also, in the presence of tea extract, the activities of ceftazidime against *S. aureus* and *E. coli* were found to be antagonistic and additive, respectively. Additive effect was also exhibited by cefotaxime against *S. aureus* and *E. coli* (Table 1 and 2).

The results of the interaction study suggest a wide disparity in the activities of these antimicrobial agents in the presence of tea. The sub-bacteriostatic concentration of tea used was to ensure that the activity obtained in the interaction study was entirely due to the antimicrobial agents. The interaction observed in this study could be attributed to the constituents of tea some of which have been shown to possess antimicrobial activity. The catechins are simple well-characterized isoflavonoids that mainly consist of epicatechin, epigallocatechin gallate, epigallocatechin and epicatechin gallate (Sakanaka *et al*., 1989). Report of an earlier study showed that epigallocatechin gallate exhibited a higher antimicrobial effect than epicatechin with *S. aureus* being more susceptible than *E. coli*. The bactericidal effect was attributed to membrane perturbation (Ikigai *et al*., 1995). The nature of this membrane perturbation and its influence on the transport of these antimicrobial agents across the cell membrane and hence their antimicrobial activity is not known. Extract and constituents of green tea have been reported to inhibit intestinal absorption of lipids in ovariectomized rats (Wang *et al*., 2006). Although this effect has been partly attributed to its inhibition of phosphatidylcholine hydrolysis (Wang *et al*., 2006). Other mechanisms may be involved which may extend this process to microorganism membranes. This suggests the likelihood of tea extract interfering with the transport of lipophilic antimicrobial

agents across the cell membranes of microorganisms which may make the lipophilicity of these agents a factor in their activity when they are combined with tea. This notwithstanding, it is doubtful if lipophilicity entirely explains the results of this interaction since we could not reconcile the lipophilicity of some of these agents and their activities with those of agents with similar solubility profile. A major important mechanism that seems to explain the results of this study is competitive binding of these agents to microorganism membrane. Epigallocatechin gallate is believed to derive its higher effect against *S. aureus,* when compared to epicatechin, from its greater binding to staphylococci (Ikigai *et al*., 1995). This suggests the possibility of constituents of tea competing with these antimicrobial agents for binding to the microorganism membrane. Although this was not assessed in this study, it seems most likely that constituents of tea may have competitively inhibited the binding of ampicillin to the staphylococci which may explain the latter's loss of activity against *S. aureus* in the presence of tea. The influence of competitive binding on the antimicrobial activity of these agents may depend on their structures and this mechanism may explain the differences in the antimicrobial activity of these agents in the presence of tea. In addition to the possible roles of lipophilicity and competitive binding in the absorption of these agents, the high elemental content of tea predisposes it to interaction with antimicrobial agents like tetracycline (Ericsson *et al*., 1982) and the fluoroquinolones (Akerele and Okhamafe, 1991; Kivisto *et al*., 1989). Tea plant reportedly contains more potash, manganese, fluorine, aluminum and selenium than other plants which may however, vary quantitatively in processed tea. It is likely that enhancement, inactivation or lowering of activity by physicochemical reactions of this nature may also account for some of the interaction of tea with these antimicrobial agents. However, if this were the case with the fluoroquinolones, it may not have applied to tetracycline which had its effect enhanced against both organisms in the presence of tea. Whatever the mechanisms are, it is evident from the results of this study that concurrent antibiotics administration and tea consumption may lead to interactions capable of reducing the antimicrobial activity of these agents.

CONCLUSION

This study has shown that there is potential for interaction when tea is consumed with antimicrobial agents. Since this is an *in vitro* study, the clinical relevance is not known but may have far reaching implications.

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