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Full Length Research Paper

In vitro study of the interaction between some fluoroquinolones and extracts of *kola nitida* seed

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The cup diffusion method (CD) was used to evaluate the *in vitro* interaction of some fluoroquinolones (ciprofloxacin, pefloxacin and levofloxacin) with extracts of *Kola nitida* seed (KNS) against a clinical isolate of *Escherichia coli*. Minimum inhibitory concentration (MIC) of the drugs was determined separately and in combination with KNS extract in ratios of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0 against *E. coli.* The result of the study revealed that the MIC of the drugs decreased when combined with KNS extract. In other words, KNS extract potentiated the effects of the fluoroquinolones against *E. coli.*

Key words: Interaction study, ciprofloxacin, pefloxacin, levofloxacin, *Kola nitida* seed.

INTRODUCTION

In rational drug therapy, the concurrent administration of two or more agents is often essential and sometimes mandatory in order to achieve the desired therapeutic goal or to treat co-existing diseases. However, the drug interaction may have different effects on the host as well as the infecting organism and can decrease potency, increase adverse effect or toxicity (Harry et al., 1998; Brooks et al., 1998; Chambers et al., 1997). A number of benefits accrue from use of combined antimicrobial therapy such as treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity in the treatment of specific infections and prevention of the emergence of resistant microorganisms (Aguwa, 1996; Hugo et al., 1993). As a result, it is highly expedient that the *in vitro* interaction of combination of antimicrobial agents be evaluated using suitable test microorganisms before such combination are clinically used (Weisser et al., 1966).

Kola nitida (Sterasliaceae), otherwise called Kola nut is widely eaten in all parts of Nigeria, both as a social food and for a host of other reasons. It is fondly called *Oji* (Igbo). *Obi-abata* (Yoruba) and *Gworo* (Hausa). *K. nitida* contains significant quantities of purine and xanthine alkaloids that are responsible for its pharmacological properties as a central nervous system (CNS) stimulant or restorative (Odimgbe, 1998). It has been used to improve chest conditions (e.g. myocardial stimulation), light sensitivity of the eye and to potentiate the action of analgesics thus affecting the cerebral circulation through its caffeine content (Nehlig et al., 1992). Kola extracts have been incorporated in pharmaceutical dosage forms as well as in many non-alcoholic cola drinks (Anozie et al., 1984).

Fluoroquinolones are a class of highly potent orally active broad-spectrum antibacterial (Davies et al., 1987), effective against both Gram-positive and Gram-negative aerobic bacteria. They target DNA gyrase and

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Table 1. Minimum inhibitory concentration (MIC) of the antibiotics and their combinations with *Kola nitida* seed against *E. coli*.

Drug-KNS ratio	CodeNo	MIC (mg/ml)		
		Ciprofloxacin	Pefloxacin	Levofloxacin
0:5	A	-	-	-
1:4	B	-	0.1719	0.1999
2:3	C	0.3200	0.3440	0.4000
3:2	D	0.4799	0.5589	0.6000
4:1	E	0.6400	0.6880	0.8002
5:0	F	0.8000	0.8600	1.0000

topoisomerase IV both of which are type II DNA topoisomerases that resolve topological constraints resulting from DNA replication and function (Drlica et al., 1997). Fluoroquinolones form a ternary complex with DNA gyrase and DNA resulting in the inhibition of DNA activities in bacteria (Drlika, 1999). In fact, some fluoroquinolones have higher affinity for topoisomerase IV than DNA gyrase. This dual inhibition pathway is responsible for the differential lethality of fluoroquinolones to different bacteria. For instance, the primary target for fluoroquinolones in *Staphylococcus aureus* and *Streptococcus pneumonia* is topoisomerase IV while for *Escherichia coli*, it is DNA gyrase (Cooke et al., 1996 and Clarridge et al., 1987). The aim of this present study is to evaluate the clinical suitability of the use of combinations of ciprofloxacin, pefloxacin or levofloxacin with *K. nitida* seed (KNS) extract against some infections of *E. coli.*

MATERIALS AND METHODS

The test organism *E. coli* (ATCC 11775) was obtained from the Bioresources Development and Conservation Programme (BDCP) Laboratory, Nsukka, Enugu State, Nigeria. Nutrient broth, nutrient agar (International Diagnostic Group, UK), Mac-Conkey agar (Merck, Germany), ciprofloxacin hydrochloride (Bayer, England), pefloxacin (M & B, England) and Levofloxacin (Hoechst, Germany) were used as obtained from their manufacturers. All other solvents and reagents were of analytical grades.

Extraction of *K. nitida* seed (KNS)

The seeds were cut into pieces, air-dried for seven consecutive days and pulverized in an end-runner mill. Approximately 500 g of the powdered seed was extracted with methanol for 18 h in a soxhlet apparatus. The extract was filtered and concentrated to a solid residue using a Gallenkamp rotary evaporator (England), under reduced pressure.

Isolation and purification of test microorganism

After collection, the *E. coli* was streaked on Mac-Conkey agar media and incubated at 37°C for 24 h to isolate pure clinical strains. Successive subculturing in nutrient broth for four days activated the microbial isolates. The activated microorganisms were subcultured into separate 5 ml sterile nutrient agar and incubated for 24 h at 37°C. The 24 h culture contained about 10^7 cfu/ml.

Standardization of microbial isolate

A 10 ml volume of sterile water was added to the agar slant containing a 24 h old culture of the purified test microorganism and shaken carefully to harvest the organism. Subsequently, dilutions were carried out to get a microbial population of 10^5 cfu/ml by comparing with Mcfarland 0.5 standard.

Preparation of drug stock solution

Stock solutions of ciprofloxacin, pefloxacin and levofloxacin were prepared by weighing and subsequent dissolution of 40, 43 and 50 mg of the drugs, respectively, in a calculated volume of double strength nutrient broth to obtain desired drug concentration. Aseptic conditions were maintained in each case. Sterile ten-fold dilutions of the stock were carried out to get the appropriate drug concentrations in 10 ml single strength nutrient broth contained in test tube.

Determination of the MIC of the drugs

A 40 mg quantity of ciprofloxacin was dissolved in 100 ml of distilled water. About 500 mg of extracts of KNS was also added to 100 ml of water. The two solutions were mixed in ascending-descending order ratios (0:5, 1:4, 2:3, 3:2, 4:1, 5:0) to obtain six corresponding preparations (A – F). Two fold serial dilutions of the respective preparations were made to yield four further dilutions. Four holes were bored in an agar plate seeded with the test organism, using sterile cork borer. Each of the dilutions was poured into a hole in the petri dish. and incubated at 37°C for 24 h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the organism. The zones of inhibition (IZD) produced by the respective concentrations of the drug against the test organism were also measured.

The procedure above was repeated for the two other drugs – pefloxacin (43 mg %) and levofloxacin (50 mg%).

Analysis of data

The interaction data were analysed statistically using bar charts.

RESULTS AND DISCUSSION

There was a decrease in the minimum inhibition concentration (MIC) observed for ciprofloxacin, pefloxacin and levofloxacin against *E. coli* with the addition of KNS (Table 1). The decrease in MIC was more pronounced as

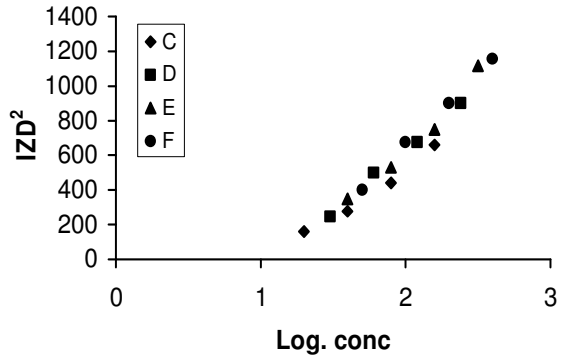

Figure 1. Log conc against IZD^2 of the interaction between ciprofloxacin and *Kola nitida* seed against clinical isolates of *E. coli*.

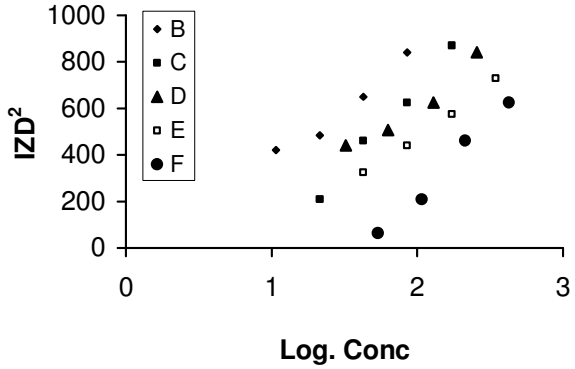

Figure 2. Log. concentration against IZD2 of the interaction between pefloxacin and *Kola nitida* seed against *E. coli*

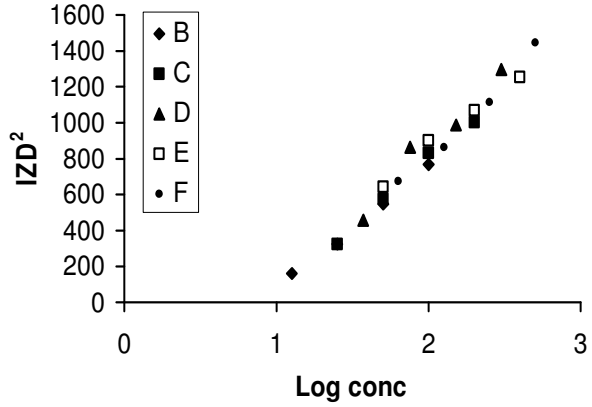

Figure 3. Log concentration against IZD2 for the interaction between levofloxacin and *Kola nitida* seed against *E. coli*.

the concentration of the KNS increased, which significantly reveals that the KNS was potentiating the effect of the fluoroquinolones. This implies that KNS increased the antibacterial activity of the antibiotics against the test microorganism – a type of synergistic or additive interaction. The combination that contained no antibiotic understandably had no zone of inhibition. A possible explanation for the observed potentiation may be a potentiation of the inhibition of the gyrase mediated DNA super coiling at concentrations that correspond with those that inhibit bacterial growth (0.1 – 10 μg/ml) (Wise et al., 1986).

The plots of log concentration of the drugs and their 2:3 combinations with KNS against the inhibition zone diameter (IZD^2) for the interactions of KNS with ciprofloxacin, pefloxacin and levofloxacin are presented in Figures 1 – 3, respectively. A close look at the figures shows that whereas the IZDs of the drugs used alone generally decreased with decreasing drug concentration, the reverse was the case with the combination where the IZDs increased with decreasing concentration of the antibiotics and their combinations. The greatest increase was however observed with levofloxacin followed by ciprofloxacin.

The fluoroquinolones are one of the new classes of highly potent and orally active broad-spectrum antibacterial antibiotics, developed from 1, 8-naphthyridine used in the treatment of respiratory tract infections, urinary tract infections (Finch, 1992; Mandell et al., 1960; William et al., 1986), prostitis (Vogel, 1995), tuberculosis (William et al., 1986), abdominal infections (Aguwa, 1996) and atypical mycobacteria infection.

The results of the study may show that concomitant intake of any of the drugs ciprofloxacin, pefloxacin or levofloxacin with KNS will lead to a potentiation of the antibacterial activities of the antibiotics. This may imply using a lower dose of the antibiotics to achieve the same therapeutic effect when the drugs are given in combination with KNS. It also implies that the intake of KNS in the form of the beverages may affect the effectiveness of a co-administered ciprofloxacin, pefloxacin or levofloxacin, as the case may be. This is obviously an example of a clinically beneficial drug-drug interaction.

In conclusion, the results so far show that *K. nitida* seed potentiated the antimicrobial effects of ciprofloxacin, pefloxacin and levofloxacin against *E. coli.* This probably suggests the possibility of concurrent use of these antibiotics and the seed in treating diseases caused by *E. coli*.

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