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The Bioenhancing Properties of *Piper guineense* Using Microbiological Assay Method

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ABSTRACT

BACKGROUND: Improving the bioavailability of drugs with the use of plant extracts with bioenhancing properties are currently on the increase. Bioenhancers modulate the activities of drug metabolising enzymes and P-glycoproteins leading to alterations in drugs' pharmacokinetic properties. The ease of development of resistance to most antibiotics is of serious concern in most parts of the world. This may be attributed to low bioavailability of some of these drugs *in vivo* resulting in sub-therapeutic concentrations of the drugs reaching the organisms and subsequently resistance development. **OBJECTIVE:** This study was designed to determine the bioenhancing properties of methanolic extract of *Piper guineense* leaves and determine its effect on the pharmacokinetic properties of pefloxacin using animal model. **METHOD:** *In vivo* studies using microbiological assay method and clinical isolates of *E. Coli* samples from hospitalized patients were used. **RESULT:** There was a significant change ($p < 0.05$) in the area under curve (AUC) of pefloxacin when co-administered with *Piper guineense* showing about 107.83 % increase from 41.50 $\mu\text{g/hr/ml}$ to 86.25 $\mu\text{g/hr/ml}$. Similarly, maximal pefloxacin concentration (C_{max}) increased from 0.9867.33 $\mu\text{g/ml}$ when pefloxacin was administered alone to 10.00 $\mu\text{g/ml}$ when administered in the presence of the extract with a significant change ($p < 0.05$) in the time to attain maximal concentration. Half life ($t_{1/2}$) and volume of distribution (V_d) of pefloxacin showed a non-significant increase and decrease respectively, in the presence of the extract. **CONCLUSION:** The co-administration of pefloxacin and extract of *Piper guineense* leaves altered the bioavailability of the drug and could be used to improve the pharmacotherapy of this agent.

Keywords: Pefloxacin, *Piper guineense*, *E. coli*, Microbiological assay, Pharmacokinetics.

INTRODUCTION

Modern researchers are increasingly shifting interest toward the improvement of bioavailability of a large number of drugs. One of these approaches is the addition of herbs with bioenhancing properties to existing formulations. A bioenhancer is an agent capable of enhancing the bioavailability and efficacy of a drug with which it is co-administered without any pharmacological activity of its own at therapeutic dose used [1]. Some molecules

such as piperine and quercetin has been successfully identified as bioenhancers and hence has been used to improve the bioavailability of some drugs. For instance, the co-administration of piperine with rifampicin lead to a reduction in the dose of rifampicin from 450mg to 200mg producing the same effect [2]. Similarly, the AUCs' of ampicillin and norfloxacin in rabbits were observed to increase by 338% and 174.6% respectively when co-administered with piperine [3]. These bioenhancers from natural compounds has been shown to act through various mechanisms such as P-glycoprotein inhibitory activity, promotion of drug absorption through increased blood flow, decreased or inhibitory effects on acid secretion thereby preventing breakdown of some drugs and inhibitory mechanisms inhibiting enzymes responsible for drug metabolisms mainly the cytochrome P450 enzymes [4,5].

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Piper guineense (PG) also known as African black pepper is distributed throughout southern parts of Nigerian especially in the rain forest zones. It has both nutritive and medicinal values and belong to the piperaceae family [6]. It is used as a medicinal agent to treat cough, cold, bronchitis, rheumatism and also used as carminative especially in gripping conditions [7,8]. It is used externally as counter irritant or as stimulating ointment and also as insecticide when pulverized. PG have been shown to contain piperine and piper-type amide alkaloid [9].

Pefloxacin is a member of the fluoroquinolones used in the treatment of bacterial infections in the different parts of the body. It is completely absorbed and is distributed throughout the body with an elimination half life of 11 hours and is excreted by the kidney. Pefloxacin has excellent tissue and body fluid penetration [10,11] and has both bacteriocidal and bacteriostatic effects, having effect against both gram positive and gram negative organisms [12]. It is extensively metabolised in the liver with the metabolite being N- desmethylpefloxacin known as norfloxacin which is also effective against bacteria [12].

The increasing utilization of complementary and alternative medicines (CAM) which incorporates the use of botanical medicines including herbs and dietary supplements either alone or in combination with prescription medicines over the last decade is now well documented. The traditional use of PG for some diseases such as cough and cold and the use of fluoroquinolones like pefloxacin for such diseases may warrant their coadministration by the patients without informing the healthcare professionals. Such combinations may lead to either positive or negative interactions which may be useful or otherwise, adversely affect drug therapy. Therefore, the effect of PG on the pharmacokinetic properties of pefloxacin when concurrently administered, forms the basis of this study.

MATERIALS AND METHODS

Collection and Identification of Plant Material

PG leaves were purchased from Nsukka market in Enugu State, Nigeria. It was identified and authenticated by Mr. Ozioko of Bioresource Development and Research centre (BDRC), Nsukka, Enugu State.

Preparation of Plant Material and Extraction Procedure

Piper guineense leaves (260 g) were dried under the shield and pulverized with a milling machine. The powder was extracted with 90 % methanol using maceration method for 48 hrs followed by filtration and then, the solution concentrated using rotary evaporator.

Drugs

Pure sample of pefloxacin were obtained as gift from Evans Nigeria Ltd and Pefloxacin tablets branded peflacine® (Fidson Nigerian Ltd) were purchased from a registered pharmacy in Nsukka.

Animals

Albino rats (100-180g) of both sexes were used in this study. They were kept separately in two cages according to their sex for one week to allow them acclimatize in their environment. Food and water were provided ad libitum. The rats serum samples were collected prior to the experiment and tested for the presence of antimicrobial using microbiological assay method to rule out antimicrobial effects of some ingested materials. The institutional and national guide for the care and use of laboratory animals was followed.

Treatment of Animals

After an overnight fast, the animals were separated into two groups (A & B) of 5 animals per group. Group A received pefloxacin (5.7mg/kg) sample alone while group B received 10mg/day of the extract for 10 days followed by the concurrent administration of the extract and the drug as in group A on the 11th day. All drugs and herbal extract doses were administered *per oral*.

Sample collection

Blood samples were collected from tail veins of the rats at 0h, 0.5h, 1h, 4h, 8h and 12h, after pefloxacin administration in both groups in heparinized containers, and centrifuged at 3000 rpm for 10minutes. The serum samples collected were used for microbiological assay.

Drug analysis

Microbiological assay [13] was employed using clinically isolated *E.coli* samples from hospitalized patients to determine the serum concentrations of pefloxacin. The Nutrient agar was supplemented with 0.1 % KH_2PO_4 , then cooled to 50 °C and inoculated with 24-h incubated *E. coli* culture (0.1 ml/100 ml agar). After the solidification, holes of 10 mm were punched out of the agar using cup borer. Subsequently the punch-holes were filled with 100 μl of serum in duplicate for calibration and samples. After the incubation at 37 °C (about 18 h), the inhibition zones were measured and the concentrations calculated.

Pharmacokinetic Analysis

Selected pharmacokinetic parameters of Pefloxacin alone and Pefloxacin with PG were determined using WinNonlin noncompartmental programme as follows: half-life ($t_{1/2}$), apparent volume of distribution (V_d), area under the serum concentration-time curve (AUC_{0-24h}), mean residence time (MRT) and body clearance (Cl), peak serum concentration (C_{max}), and time to reach maximal concentrations (t_{max}). Independent t-test was used to determine the significant difference between the two groups using SPSS version 16.

RESULTS

Table 1 shows the comparative pharmacokinetics of pefloxacin, administered alone and in the presence of PG while figure I shows their concentration time graphs. Pefloxacin maximum concentration of 7.33 $\mu\text{g/ml}$ occurred at 1.17 hr when

administered alone and occurred at 3.33 hr in the presence of PG with maximal conc of 10.00 µg/ml.

Table 1: The pharmacokinetic parameters of Pefloxacin administered alone and when co-administered with *Piper guineense* using microbiological assay.

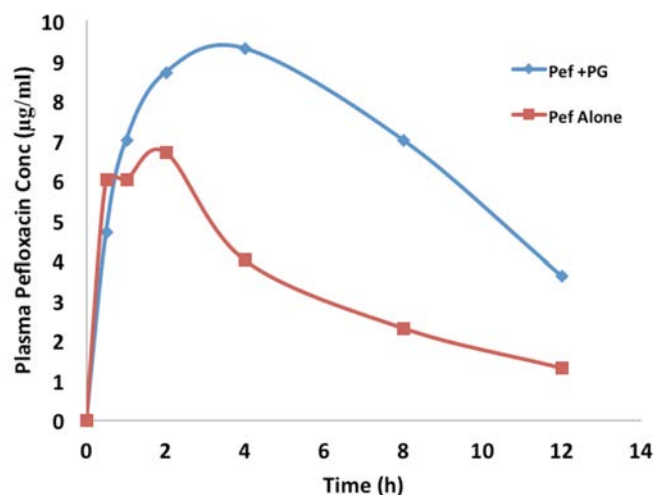
PARAMETER	PEF ALONE	PEF + PG
T_{max} (hr)	1.17 ± 0.441	3.33 ± 0.667*
C_{max} (µg/ml)	7.33 ± 0.667	10.00 ± 1.000
C_{last} (µg/ml)	1.33 ± 0.333	3.67 ± 1.667
AUC(µhr/ml)	41.50 ± 5.246	86.25 ± 14.297*
$t_{1/2}$ (hr)	5.45 ± 1.594	7.27 ± 0.180
V_d (ml/kg)	2.79 ± 0.219	2.10 ± 0.400
Cl (ml/kg/hr)	0.40 ± 0.084	0.20 ± 0.043
AUMC(µg/ml/hr ²)	180.67 ± 42.686	456.42 ± 98.906
MRT (hr)	4.47 ± 0.52	5.53 ± 0.368

*P<0.05

Pef is pefloxacin, PG is *Piper guineensis*, T_{max} is time taken for drugs to attain maximal plasma concentration. C_{max} is maximal drug plasma concentration, C_{last} is last measurable drug plasma concentration, AUC is area under curve from the time of dosing to the time of the last observation, AUMC is area under moment curve from the time of dosing to the time of last measurable concentration, MRT is mean residence time, $t_{1/2}$ is terminal half-life, V_d is volume of distribution based on the terminal phase, Cl is total body clearance.

There was also a significant increase in the AUC of pefloxacin from 41.50 µg/hr/ml when administered alone to the animals to 86.25g/hr/ml when concurrently administered with PG increasing the AUC of the co-administered angets by 107.83 %. The half life ($t_{1/2}$) of the drug increased non significantly from 5.45 hr when pefloxacin is administered alone to 7.27 hr in the presence of the extract while volume of distribution (V_d) of the drug decreased from 2.79 ml/kg to 2.10 ml/kg in the presence of the extract. Similarly, clearance (Cl) showed a slight decrease from 0.40 ml/kg/hr to 0.20 ml/kg/hr in the presence of the extract. The area under moment curve (AUMC) of pefloxacin showed an appreciable increase (152.67%) from 180.67 µg/ml/

Figure 1: Effect of *Piper guineense* on plasma concentration of Pefloxacin (Pef)



Pef is Pefloxacin and PG is *Piper guineensis*

hr² to 456.42 µg/ml/hr² in the presence of the extract where as, mean resident time (MRT) showed about 19.46 % increase from 4.47 hr to 5.34 hr.

DISCUSSION

Bioavailability according to Toutain and Bousquet-Melou is the proportion of a drug which is absorbed and available to produce systemic effect and, it is a key pharmacokinetic parameter which expresses the proportion of a drug administered by any nonvascular route that gains access to the systemic circulation [14].

The term bioenhancing activity is also defined as a substance at lower dose level, which in combination with a drug or nutrient provides more availability of the drug by reducing the dose of the drug or nutrient resulting in enhanced efficacy of such drug(s) [15]. Bioenhancers are of therapeutic and/or economic importance since the co-administration of a bioenhancer could lead to the reduction in drug dose volume with ultimate good response. Hence, Varshneya defined bioenhancers as substances which when mixed with drug enhance the effectiveness of the drug without taking away its properties and therefore has the advantages of reducing drug dosage and minimizing the dangers of drug resistance and also, that reducing the drug dosage will minimize drug toxicity [16].

Piperine a major alkaloid constituent present in most spices belonging to the piperaceae and zingiberaceae family has been identified as a bioenhancer [17]. Piperine exhibit both nutritional and medicinal properties including anti-inflammatory, antifungal, antioxidant and hepatoprotective properties and reduction of gastro intestinal transit time among others [18,19,20]. Piperine as a bioenhancer has been shown to prolong the AUC of pheytoin and adding piperine to anti-TB

and leprosy drugs have been found to enhance their effectiveness with the dose of the anti-TBs being reduced to half, to produce the same effect as with the full dose [16].

From the result of this study, leaves extract of PG has been shown to exhibit bioenhancing properties by enhancing the extent of pefloxacin absorption. This enhanced pefloxacin absorption can be seen from the higher values of C_{max} and AUC of the drug when co-administered with the extract. This enhanced absorption may be due to the piperine constituent of PG. Piperine has been shown to be the main amide active in *Piper guineensis* [21] and piperine has been shown to be a bioenhancer by many researchers increasing the bioavailability of different agents. Furthermore, this enhanced absorption and systemic bioavailability of the drug in the presence of the extract may be due to possible suppression of drug metabolising enzymes by the extract. The possible suppression of drug metabolizing enzymes are further indicated by the 33.39 % prolongation of the drug's half life ($t_{1/2}$) when administered with the extract. This was further indicated by the decreased clearance of the drug and also, slightly improved MRT.

The metabolism of pefloxacin a member of the fluoroquinolone is mediated by the cytochrome P450 enzymes in the liver mainly CYP 1A2. Fuhr et al pointed out that CYP 1A2 is the only enzyme with a relevant contribution to pefloxacin N-4 demethylation [22] which is responsible for metabolism of pefloxacin. However, PG extract can be a good inhibitor of this enzyme since it has been shown to contain piperine that has been established to be a non specific inhibitor of drug metabolising enzymes which shows little discrimination between different cytochrome P450 forms [23]. Furthermore, the slight decrease in the clearance of the drug in the presence of the extract is further suggestive of possible metabolising enzyme inhibition.

The limitations of this work include the inability of the researchers to isolate and quantify the piperine constituent of this extract. However, this together with the elucidation of the various suggested mechanisms of activity forms the basis of our future research.

CONCLUSIONS

Both the rate and extent of pefloxacin absorption were improved by its coadministration with extracts of PG leaves as seen from the alterations in the pharmacokinetic properties. Our result is similar to the result of Dama et al who reported increase in AUC, AUMC, MRT and in general, the bioavailability of pefloxacin in the presence of piperine [24]. This could be used clinically in practice to improve the pharmacotherapy of this agent, a practice that could be achieved through a reduction in drug dosage and therefore, reduction in the drug toxicity and cost.

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