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Impact of Yoyo bitters® on the Pharmacokinetic Profile of Ciprofloxacin in Healthy Nigerian Volunteers

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ABSTRACT

Ciprofloxacin and Yoyo bitters® (a liquid herbal oral preparation) are readily and widely available as prescription drug and herbal medicine, respectively in Nigeria. They are taken separately or concomitantly. The high metal content of Yoyo bitters poses to be a potential problem for the bioavailability of fluoroquinolones. Thus, this study was carried out to investigate the possible drug-herb interaction between Ciprofloxacin tablet and Yoyo bitters when co-administered. Pharmacokinetics of oral single dose ciprofloxacin (500 mg) tablet was evaluated in 30 healthy volunteers. The study design was an open, single dose randomized two-way comparative crossover treatment groups. Each group either took ciprofloxacin (500 mg) tablet with 200 mL of water or ciprofloxacin with 30 mL of yoyo bitters. A wash out period of two weeks was observed, and the treatment groups were interchanged. Blood samples were collected and analyzed for ciprofloxacin in plasma using a modified High-Performance Liquid Chromatographic (HPLC) method with UV detector at 278nm. Non-compartmental analysis was employed for pharmacokinetic parameters estimation. Results indicated that Yoyo bitters altered the Pharmacokinetics of ciprofloxacin (Cmax, AUC, Vd, Tmax, $t^1/_2$, Kel and Kab) when administered concomitantly, although not statistically significant ($p>0.05$). Therefore, for the desired therapeutic effect of ciprofloxacin to be optimally achieved, concomitant administration with Yoyo bitters or any herbal preparation should be staggered.

Keywords: Ciprofloxacin, Drug-Herb interaction, Pharmacokinetics, Yoyo bitters®

Introduction

Herbal medicines have emerged as a common choice therapy for self-care among individuals and are now taking a more active role in users' healthcare.[1] In addition, irrational claims or advertisements by manufacturers through different mass media have enhanced the wide spread use of herbal medicines among the general population in developing countries.[2] Most of the time, the herbal medicines are sold as non-prescription medicine and the patients use it along with the prescribed conventional medicine at their own risks.[3] Herb-drug interaction is an important factor to be investigated because there is always a chance to get undesirable therapeutic effect of the prescribed allopathic medicine.[4-6]

Ciprofloxacin is a widely used antibiotic of the class, fluoroquinolones that is active against numerous pathogens.[7] It is rapidly and well absorbed from the gastrointestinal tract, widely distributed in the body with good tissue penetration.[8,9] However; its oral absorption can be affected negatively, by common concomitant administration of agents containing metal cations.[10,11] Studies with quinolones have revealed metal-drug interactions.[12] Ciprofloxacin has been observed to exhibit decreased absorption when co-administered with polyvalent cations such as aluminum, calcium, copper, iron, magnesium, manganese and zinc found in dairy products (milk, cheese and yogurt), antacids and mineral supplement.[13] The same interaction has been shown to occur when calcium-fortified orange juice is taken at the same time as ciprofloxacin.[14] The decreased bioavailability was presumed to be as a result of formation of poorly absorbed metal ion-ciprofloxacin complexes.[15,16]

Yoyo bitters® is a galenical oral preparation made from a blend of various parts and fruits of plants such as *Aloe Vera*, *Cinamum aromaticum*, *Citrus aurantifolia*, *Acinos arvensis and Chenopodium murale*. It has satisfied the requirements laid down by the National Agency for Food and Drug Administration and Control (NAFDAC) for the registration of herbal Medicines. The formulation is indicated for the treatment of kidney and bladder infections, normalize intestinal movement, help regulate blood pressure, facilitate digestion and to control body weight.[17]

Herbal medicines have been found to also contain metallic cations[18,19] which could lead to herb-metal interaction. Some herbal medicines share the same drug metabolizing enzymes and drug transporters with several conventional medicines, there is bound to be herb-drug interaction which is an important factor to be investigated for assessing and minimizing clinical risk. Typical examples of herb-drug interaction and observed effects include: bleeding when warfarin is combined with ginkgo (*Ginkgo biloba*), garlic (*Allium sativum*), decreased bioavailability of digoxin, theophylline, cyclosporine when these drugs are combined with St John's wort.[20-22] It was also reported in a study that Yoyo bitters inhibited the metabolism of Paracetamol tablet in human subjects.[23]

This study was aimed at examining the metal content of Yoyo bitters and to investigate the possible drug-herb interaction of Yoyo bitters on

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the pharmacokinetics profile of oral single dose ciprofloxacin in human volunteers.

Materials and Methods

General Experimental Procedure

Ciprofloxacin reference standard and Gatifloxacin internal standard were kindly provided by Shreechem Pharmaceutical Nig. Ltd and Tamaflex, (a brand of ciprofloxacin) from Tamar and Pharez Pharmaceutical Nig. Ltd, Kano, manufacture date: 07, 2015; expiry date: 06, 2018; batch number MTFT-1501 while Yoyo bitters from ABBLAT Nig. Company Ltd, manufacture date: June 2016; expiry date: June 2018; batch number: NBR1520 was purchased from a registered retail pharmacy outlet in Mushin, Lagos.

Acetonitrile (HPLC grade) and Phosphoric acid were purchased from Merck, Germany and all other reagents were of analytical grade.

An Atomic Absorption Spectrophotometer (Thermo AA series) was used to assess the cation (Na, K, Ca, Mg, Fe, Zn, Cu, Cd, Mn, Pb and Cd) content of Yoyo bitters. The sample was digested with an acid mixture consisting of HNO_3: $HClO_4$ (9:1).[24,25]

The High-Performance Liquid Chromatography (HPLC) system is an Agilent technologies series 1200 consisting of a UV detector, an auto sampler, a quaternary pump and a reversed phase C-18 column (4.6 mm × 250 mm, 5 µm Zorbax) was employed for the quantification of ciprofloxacin.

Participants' treatment, Sampling time and collections

This study was approved by the Health Research Ethics Committee of Lagos University Teaching Hospital (ADM/DCST/HREC/APP/048). Thirty (30) healthy volunteers participated in a single oral dose and randomized two-way crossover study. The volunteers mean age was 34.7 ± 7.5 years and mean weight was 75.8 ± 9.0 Kg.

All participants received both verbal and written information on the study and were given written informed consent prior to participation. All participants were non-smokers and not on any medications. They also abstained from coffee, grape fruits, antacids, multivitamins cimetidine, green tea, food supplements, beverages or drug that can affect ciprofloxacin four weeks before investigation. Consent forms were signed, and we got verbal assurance from the volunteers who were members of CMUL/LUTH community.

All participants were in good health as indicated by medical history and routine physical examinations. The volunteers were regularly monitored during the experimental period for the development of any possible adverse effect.

First phase

The volunteers were divided into two groups. Each group was either administered with 500 mg single oral dose ciprofloxacin tablet with 200 mL of water or ciprofloxacin 500 mg with 30 mL of Yoyo bitters. Venous blood sample (5 mL) was collected into lithium heparin bottles immediately before (0 min) and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6,8,10, 12, 24 and 48 h after oral administration of ciprofloxacin tablets. Blood samples were centrifuged at 4000 x g for 10 mins and supernatant plasma was collected into another pre-labeled tube and stored at -20°C until analysis.

Second phase

After a 2-weeks wash out period, the participants group were interchanged, those that took 500 mg ciprofloxacin with 30 mL of yoyo bitters before now received 500 mg single oral dose ciprofloxacin tablet with 200 mL of water. Blood sample were then collected as in the first phase.

HPLC Analysis of Ciprofloxacin

The concentration of ciprofloxacin in the plasma was determined by HPLC assay method developed by Norman *et al*[26] with minor modifications. For the quantification, 1 mL of plasma was added to 0.1 mL internal standard, gatifloxacin and 0.9 mL of 10% perchloric acid. The mixture was vortex for 15 mins and then centrifuged at 4000 x g for 15 mins. The supernatant was collected into another tube. 20 µL of this supernatant was injected into the HPLC.

The HPLC mobile phase consists of 15% acetonitrile and 85 % 0.025 M phosphoric acid (pH 3.0) adjusted with triethylamine.[27] The flow rate was set at 1.5 mL/min and detection was performed at 278 nm. The mobile phase was filtered through 0.45 µ Millipore membrane filter paper. The method was validated using correlation, linearity, precision and recovery rule. The calibration curves were linear over the ranges 0-10 µg/mL with correlation coefficient (r^2) of 0.995. The limit of detection was 50 ng/mL. The coefficient of variation for assessment of precision within and between days was less than 6%.

Pharmacokinetic Analysis

The Pharmacokinetic parameters such as maximum drug concentration (Cmax), time to maximum concentration (Tmax), elimination half-life ($t^1/_2$), elimination rate constant (Kel), absorption rate constant (Kab), area under plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$), apparent volume of distribution (Vd), total oral clearance (Cl/F) and mean residence time (MRT) were calculated by non-compartmental model[28] using WinNonlin Professional®PK modeling tool version 2.1 Pharmacokinetic software.

Statistical Analysis

Statistical analysis was done using Origin 6.0 software. Comparisons of pharmacokinetic parameters were done using t-test, in all cases, a value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Assessment of Cation content of Yoyo bitters®

Concentrations (ppm) of 10 metals cations contained in the Yoyo bitters were determined to be 48.8, 11.63, 12.0, 3.2, 1.32, 0.05, 0.11, 0.21, 0.27 and 1.5 for Na, K, Fe, Cd, Cu, Mn, Ca, Mg, Zn and Pb, respectively.

Pharmacokinetic Parameters

The mean pharmacokinetic parameters of ciprofloxacin alone after a single dose and with co-administration of Yoyo bitters are listed in Table 1 while the mean plasma concentration-time profile plot of ciprofloxacin and that of the concomitant oral administration of Yoyo bitters are shown in Figure 1.

As demonstrated in this study, Yoyo bitters contained a large amount of calcium (12 mg/L), magnesium (3.2 mg/L), lron (1.32 mg/L), zinc (0.05 mg/L), manganese (0.27 mg/L) and copper (0.11 mg/L). These metal cations were the ones accounted for in Yoyo bitters and the chelation of these metals with Ciprofloxacin had been well documented.[28-31] These metal cations present are sufficient to trigger a reduction in the ciprofloxacin absorption profile hence, the lower Ciprofloxacin absorption observed is expected.

Zhu *et al*.,[18] reported an increase in lipophilicity of Ciprofloxacin when complexed with metal ions. This statement was attributed to enhancement and tissue uptake as observed in their report. Similarly, an increase of 27% in apparent volume of distribution was observed in our study signifying increase in drug tissue distribution.

The trend of the data obtained for the pharmacokinetic parameters are similar to previous work done.[18,19,32,33] Alterations were observed from the profile when ciprofloxacin was administered concurrently with yoyo bitters.

The Cmax was found to be 2.55 ± 1.48 mg/L for ciprofloxacin alone and 2.40 ±1.15 mg/L for concurrent administration of ciprofloxacin and yoyo bitter. In particular, the Cmax of ciprofloxacin was reduced by 6%. A decrease in Cmax which is the maximum concentration of the drug achieved in the plasma, means that the onset of the therapeutic action is affected. However, the increase was not statistically significant ($p = 0.80$).

The time taken to attain peak concentration, Tmax, for those who took ciprofloxacin alone was 2.11 h and 1.89 h when ciprofloxacin was administered with yoyo bitters. The shorter the Tmax; the faster the rate of absorption. It was observed that yoyo bitters decreased the Tmax of ciprofloxacin, hence increased the rate of absorption (Kab) of ciprofloxacin into systemic circulation as justified by the Kab (1.28 h^{-1} and 1.39 h^{-1}) for ciprofloxacin alone and ciprofloxacin with yoyo bitters, respectively. Also, the difference was found to be insignificant ($p > 0.45$). Increase in rate of absorption leads to decrease in onset of action and increase in peak plasma concentration. However, this was contrary to our result where the peak plasma concentration was reduced. The elimination rate constants Kel were found to be 0.11 and 0.08 for ciprofloxacin alone and ciprofloxacin with yoyo bitter concurrently.

Table 1: Pharmacokinetic Profile of oral Ciprofloxacin 500mg after a single dose and with concomitant administration of Yoyo bitters.

Pharmacokinetic Parameter	Ciprofloxacin Alone	Ciprofloxacin + Yoyo bitters	P-value
Cmax (mg/L)	2.55 ± 1.48	2.40 ± 1.13	0.80
Tmax (h)	2.11 ±0.60	1.89 ± 0.60	0.45
Kel (h^{-1})	0.11 ± 0.06	0.08 ± 0.07	0.45
$T_{1/2}$ el(h)	5.97 ± 2.35	8.86 ± 5.01	0.36
K_{ab} (h^{-1})	1.28 ± 0.67	1.39 ± 0.79	0.78
$T_{1/2}$ ab(h)	0.78 ± 0.71	0.75 ± 0.55	0.91
$AUC_{0-\infty}$ (mg L^{-1} h)	4.39 ± 5.48	2.97 ± 2.84	0.52
Vd (L Kg^{-1})	24.45 ± 5.47	45.35 ± 5.80	0.09
Cl/F (Lh^{-1} kg^{-1})	2.49 ± 2.87	2.74 ±3.75	0.76
MRT (h)	1.86 ± 1.41	2.48 ± 2.66	0.56

values are expressed as mean ± Standard Error of Mean (SEM), n = 30. Cmax= maximum drug concentration, Tmax = time to maximum concentration, $t^1/_2$ = elimination half-life , Kel = elimination rate constant, Kab = rate of absorption constant, $AUC_{0-\infty}$ = area under plasma concentration-time curve from zero to infinity, Vd = apparent volume of distribution, Cl/F = total oral clearance, MRT = mean residence time.

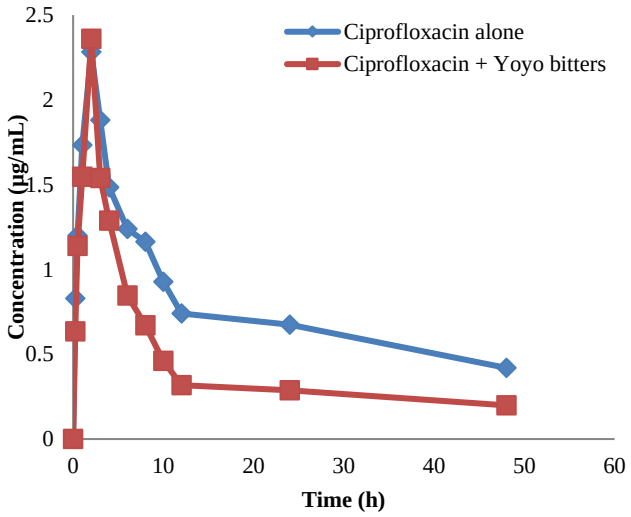

Figure 1: Plot of mean plasma concentration of oral single dose ciprofloxacin 500mg alone and concomitant administration of Yoyo bitters with ciprofloxacin against time.

The Elimination half-lives ($t_{1/2}$) were found to be 5.97 h and 8.86 h for ciprofloxacin and ciprofloxacin-yoyo bitters, respectively. These values were found to be longer with ciprofloxacin and yoyo bitter concurrently. This increase; though not statistically significant ($p > 0.05$) showed that yoyo bitters delayed the clearance of ciprofloxacin from plasma which means longer duration of action. This observation is similar to the report of Kumdi *et a*l, where yoyo bitters increases the elimination half-life of paracetamol.[34] The Area under the curve ($AUC_{0-\infty}$) was found higher for ciprofloxacin alone (4.39 $mghL^{-1}$) than when ciprofloxacin was taken with yoyo bitter (2.9 mgh L^{-1}). This means the bioavailability of ciprofloxacin will be reduced in the presence of yoyo bitters and consequently resulted to a reduction in therapeutic efficacy of ciprofloxacin,[3,4] though the result is not significant ($p = 0.52$).

There was an increase in Apparent Volume of distribution (Vd) of ciprofloxacin alone from 24.45 Lkg^{-1} to 45.35 Lkg^{-1} when ciprofloxacin was administered with yoyo bitters. Even though, yoyo bitters certainly influence the increase in drug distribution of ciprofloxacin, it is not statistically significant.

The total oral clearance (Cl/F) obtained for ciprofloxacin alone was 2.49 $Lh^{-1}kg^{-1}$ and 2.74 $Lh^{-1}kg^{-1}$for ciprofloxacin with yoyo bitters which implies that yoyo bitters promote the metabolic clearance of ciprofloxacin. This result is at variance with elimination half-life obtained although the increase was found to be insignificant.

The Mean Residence Time (MRT) was also found higher in ciprofloxacin with yoyo bitters than in ciprofloxacin alone which implies that systemic clearance of ciprofloxacin was affected by yoyo bitters. The presence of yoyo bitters caused a lowering of total clearance of ciprofloxacin.

Conclusion

The use of herbal products has been on the increase in most developing countries. There has been recommendation on the need to implement increased public awareness and educational programs on the use of herbal medicines, stressing the public health consequences of drug-herb interactions. Current data showed that co-administration of Yoyo bitters has led to altered pharmacokinetic parameters of ciprofloxacin, although the effect is not statistically significant. There is a need to take greater caution on co-administration of the two formulations. Staggering of the time of administration might be a better option.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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