Palestinian Medical and Pharmaceutical Journal (PMPJ). 2016; 1(1): 1-6

Influence of *Corchorus Olitorius* (Molokhia) Soup on the *In Vivo* Absorption of Ciprofloxacin from Immediate Release Tablet in Rabbits

Asma Radwan[1], Naser Shraim[1], Josephean Elaraj[1], Malak Jada'a[1], Anwar Alheen[1], Yasmeen Khanfar[1], Nidal Zatar[2], Mohamad Abdaldaym[3]*

[1]Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. [2]Department of Chemistry, Faculty of Science, An-Najah National University, Nablus, Palestine. [3]Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus, Palestine.

*Corresponding author: asma.radwan@najah.edu

Received: (24/5/2016), Accepted: (23/8/2016)

ABSTRACT

This study was undertaken to evaluate the effect of *Corchorus Olitorius* (Molokhia) soup on the oral absorption of ciprofloxacin from solid dosage forms in rabbits. A single dose of ciprofloxacin tablet (125 mg) was administered to rabbits in a fasted state or with Molokhia soup. Plasma ciprofloxacin concentrations were measured by high-performance liquid chromatography. The results demonstrated a profound effect of Molokhia soup on the bioavailability of ciprofloxacin compared to the fasted state. The intake of Molokhia soup reduced the rate and extent of ciprofloxacin oral bioavailability. The geometric mean (C_{max} and AUC) values of ciprofloxacin were significantly lower under fed compared to the fasted states. Administration of ciprofloxacin with Molokhia soup was associated with a delay in the time to reach peak concentration (T_{max}) by about half hour. Therefore, co-administration of Molokhia soup with ciprofloxacin may have a potential negative effect on the release and oral absorption of Ciprofloxacin from tablets, This may result in serious decrease in the efficacy of this antibiotic and potential bacterial resistance.

Keywords: Corchorus Olitorius, Ciprofloxacin, Absorption, Food Effect.

INTRODUCTION

Oral drug administration is the most convenient route for drug delivery because of its feasibility and safety. The oral bioavailability of drugs may be affected by presence of food in the gastrointestinal tract. Food may lead to alterations in the pharmacokinetics or pharmacodynamics of the active pharmaceutical ingredients. Meals may increase or decrease or have no effect on the absorption of drugs. Undesirable food effects may result in sub-therapeutic levels of the drug and a high risk of treatment failure (1).

The proposed theories explaining the possible mechanisms behind undesirable food effects include: Food induced changes in the GI physiology (pH, motility, transient times, and viscosity), chelation interactions of the drug with food components, delayed tablet disintegration and drug dissolution rates under fed conditions (2-7).

Ciprofloxacin is a fluoroquinolone antibiotic which is effective against many gram-positive and gram-negative bacteria. It is commonly prescribed for the treatment of urinary tract infections.The bactericidal action of this drug is due to its interference with the DNA gyrase enzyme, which is involved in the synthesis of bacterial DNA (8).

The literature data is conflicting regarding the assignment of ciprofloxacin to a specific Biopharmaceutic Classification System (BCS) class. Some studies classified ciprofloxacin as BCS II/IV (9). Breda and co-authors characterized it as BCS II (10).On the other hand, other studies considered it to lay between class II and III since its solubility is pH dependent, that is, its solubility is high at low pH and low at basic pH (11).

Pharmacokinetic studies on ciprofloxacin have been conducted in humans and many animal species. Ciprofloxacin is well

absorbed after oral administration and has good oral bioavailability (70% and 80%). The serum half-life of ciprofloxacin is about 4-6 hour (hr), with the maximum plasma concentration C_{max} within T_{max} 1 to 2 hr (12-14).

A previously conducted study showed that concomitant intake of food with ciprofloxacin has no significant effect on the extent of drug absorption, except a delay in the rate of its absorption represented by prolonged T_{max} (15). However, the oral bioavailability of Ciprofloxacin was significantly reduced with the intake of dairy products due to chelation interaction between the drug and the di-and trivalent cations (16, 17).

Corchorus Olitorius is a member of the Tiliaceae plant family that is commonly cultivated in Egyptian and Middle Eastern countries. It is also known as mulukhiya, molohiya, mloukhiya and jute. It has many therapeutic effects like diuretic, antipyretic, analgesic, antimicrobial, antitumor and antioxidants activity (18). Furthermore, *C. Olitorius* is a good source of vitamins (C, E, K, B6, A) and minerals such as potassium, iron, calcium, magnesium, phosphorous, and selenium. The leaves of this plant are rich in mucilage polysaccharides and hydrocolloids (19). *C. Olitorius* leaves are prepared as a viscous soup, and is considered as one of the most popular and delicious dishes in the Mediterranean, Arab and other worldwide regions.

The effect of Molokhia soup on the pharmacokinetics of ciprofloxacin has not been studied before. The aim of this study is to evaluate the effect of Molokhia soup on the bioavailability of a single dose of ciprofloxacin in rabbit models and to provide a mechanical explanation of food effects.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride immediate release tablets (Ciprocare) ® 250 mg (Pharmacare, Palestine) were used in this study. Ciprofloxacin hydrochloride powder (purity 99%) was donated kindly by (Pharmacare, Palestine). High Performance Liquid Chromatography (HPLC) grade solvents of acetonitrile (ACN), triethylamine, phosphoric acid (85%), were purchased from Sigma-Aldrich. Molokhia leaves were obtained from local market.

Molokai soup was prepared by cutting the fresh leaves of *C. olitorius* (50 g) and mixing thoroughly with 1 L water using mixer and heating until boiling.

Animals

Eight local breads rabbits, weighing between (3-4 kg), were included in this study. The animals were kept indoors and fed with fresh green fodder. Water was provided freely as much they required. The experimental protocol was done under internationally accepted animal welfare guidelines.

In vivo bioavailability

***Protocol of the Study*:** The effects of Molokhia soup on the pharmacokinetics of Ciprofloxacin was studied after administration of an oral single dose in healthy rabbits.

Study design: In a parallel design**,** eight rabbits were randomly divided in to two groups (n=4). They had not been previously fed any food containing antibiotics especially ciprofloxacin and they were in fasting state overnight before drug administration. All animals were exposed to similar conditions.

Drug administration: Each rabbit in the first group was fed with 5 mL of Molokhia soup in the morning, whereas the rabbits in the second group were in a fasting state at the time of drug administration. Each rabbit received a single oral dose of 125 mg of the drug. Ciprocare® 250 mg tablets in its solid dosage form were broken in half and administered orally to the eight rabbits. The drug was administered within 5 min after soup intake.

Blood sampling: About 2 ml blood samples were withdrawn from the rabbits ears in heparinized glass tubes using sterilized plastic syringes at times: 0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.5,2, 3, 4 and 10 hrs after the drug administration. The blood samples were immediately centrifuged using (Hettich centrifuge universal 320, United Kingdom) at 3000 rpm for 10 min to separate the plasma, which were stored at -20°C until analyzed.

Sample preparation: A liquid-phase extraction was carried out by adding 600 micro liter (µL) of acetonitrile to 200µL of plasma containing Ciprofloxacin in a 5-mL glass tube to precipitate protein and the tubes were vortexed for 2 min. The samples were centrifuged at 4000 rpm for 10 min, and then the upper organic phase was evaporated to dryness overnight in oven (memmert, HongKong) set at 40°C. The residue was dissolved in 100 µL of the HPLC mobile phase, in order to be injected into the HPLC system.

HPLC analysis: The plasma concentration of Ciprofloxacin was determined using high performance liquid chromatographic (HPLC) procedure as described by Kordick and co-authors with some modifications (20).

The Breeze HPLC experimental conditions were optimized on stainless steel column 75 x 4.6 mm ID packed with base deactivated octadecylsilyl silica gel chromatography (5µm). The mobile phase used was a mixture of 0.025 M phosphoric acid and acetonitrile (87:13).The pH of phosphoric acid was adjusted with triethylamine to 3.0. The flow rate used was 1.5 mL/min and the injection volume was 10 µL. The analyte was detected with a UV detector at wavelength 278 nm.

Calibration curve for Ciprofloxacin in mobile phase, in the concentration range of 0.2 and 150 µg/mL, was constructed. Afterwards, the unknown plasma levels of ciprofloxacin were quantified by comparison of ciprofloxacin peak area with a calibration curve formula using Microsoft Office Excel 2007. The stock standard solution was prepared by adding 12.5 mg of ciprofloxacin HCl, accurately weighed, to 25-ml volumetric flask, then adding 0.1 mL of 7% phosphoric acid.

Pharmacokinetic Analysis

The plasma concentration versus time curve for each individual rabbit was fitted using PKSolver- a freely available add-in program for Microsoft Excel. Pharmacokinetic parameters were determined using non-compartmental analysis. Peak plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}) values were obtained from observed data on the Ciprofloxacin plasma concentration – time curve for each individual rabbit. Elimination or terminal rate constant (k) was calculated from the terminal portion of the plasma concentration – time curve using least-square regression analysis of the logarithm of concentration versus time.

Biological half-life ($T_{1/2}$) was calculated by the following relationship:

$$\mathbf{T_{1/2} = 0.693/k}$$

The area under concentration curve (AUC) was calculated by trapezoidal rule to 10 hours.

Statistical Analysis

Data management and analysis were performed using Microsoft Office Excel 2007. Data from pharmacokinetic analysis are reported as mean ± SEM (n=4). Student`s t-test was used to evaluate the significance of difference between the means of kinetic parameters obtained from the 2 groups. P-values <0.05 were considered significant.

RESULTS

The retention time for ciprofloxacin was 4 minutes with limit of detection (LOD) of 0.06 µg/mL and limit of quantification (LOQ) of approximately 0.2 µg/mL.

The linearity of the calibration curve for Ciprofloxacin was studied at the concentration range of 0.2-150 µg/mL. The standard curve showed a good linearity over the range concentrations examined. The equation that best described the relationship between ciprofloxacin concentration levels and response is shown as:

$$\mathbf{Abs = 0.0273\ Conc. - 0.0709,\ R^2 = 0.9977}$$

***In vivo* bioavailability study**

The mean ± SD plasma concentration of Ciprofloxacin versus time obtained following oral administration of Ciprocare® half tablet (125 mg) to 2 groups of rabbits are plotted in figure 1.

The results show a significant effect of Molokhia soup on the bioavailability of ciprofloxacin. The median T_{max} was 1.5 hr under fasting conditions compared to 2 hr under fed condition. The geometric mean

value for C_{max} was lower when ciprofloxacin was co-administered with Molokhia soup. Moreover, the AUC was significantly reduced after administration of Molokhia soup.

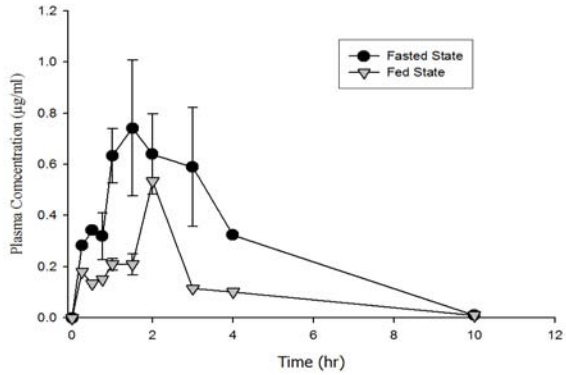

Figure (1): Plasma concentration of Ciprofloxacin in rabbits with/ without Molokai soup.

The pharmacokinetic parameters of ciprofloxacin absorption in each group are listed in Table 1 along with the statistical significance values. The elimination half-life of ciprofloxacin in rabbits was 3.05 hr. This value for half-life is in accordance with previous studies (half-life 3-6 hour) (12-14).

Table (1): The pharmacokinetic parameters for Ciprofloxacin HCl under fasted and fed conditions.

	Treatment	
	Fasting State	**Fed State**
T_{max} (hr)	1.75 ± 0.288	2.00 ± 0.707
C_{max} (µg/mL)	0.792 ± 0.286	0.452± 0.092
AUC (µg.h/mL)	2.642 ± 0.488	0.964 ± 0.228

DISCUSSION

This study was conducted to determine the effects of Molokhia soup on the rate and extent of absorption of ciprofloxacin antibiotic in rabbits, and to provide explanations for the observed effects of food.

Using the ratio of the means of AUC_{0-10} and C_{max} for Ciprocare® Tablets in fed and fasting studies for the bioequivalence comparison, it was found that fed study had significantly lower bioavailability compared to that in fasting study. The ratios for AUC_{-Fed}/ $AUC_{-Fasting}$ and $C_{max-Fed}$/ C_{max}-$_{Fasting}$ were 0.36 and 0.57, respectively.

The ingestion of Molokhia soup has significant effect on the *in vivo* absorption of Ciprofloxacin in rabbits. The maximum drug concentration C_{max} was significantly reduced by the intake of molokhia soup by 30% compared to the fasted state *(P* value less than 0.05). The absolute bioavailability of ciprofloxacin was significantly lower when the drug was co-administered with Molokhia soup (43% reduction).

Ciprofloxacin was rapidly absorbed when given orally as a solid dosage with a T_{max} of 1.75 h. Under fed conditions, T_{max} was prolonged compared with drug administration under fasted state (2 hr). However, this effect was statistically insignificant with *P* value greater than 0.05.The observed delay in T_{max} under fed state is in accordance with previous reports, which demonstrated a prolonged T_{max} under fed state.

Meal viscosity is one of the factors contributing to the undesirable food effects. *C. Olitorius* leaves are rich in hydrocolloids and mucilage, its viscosity was reported to surpass that of guar gum and locust been gum under similar conditions. The high content of these polysaccharides in plant leaves explain the viscous consistency of this soup. The observed findings can be attributed to high viscosity of Molokhia soup, which may be another possible explanation for these observations, which is the formation of film layer on surface of the tablet as a result of Molokhia leaves precipitation on the tablet.

The observed undesirable food effects may have significant clinical outcomes. Molokhia ingestion can definitely decrease ciprofloxacin levels in the blood and this may lead to treatment failure and bacterial resistance. Therefore, ciprofloxacin is recommended to be administered on an empty stomach at least one hr before or 2 hr after Molokhia soup intake.

CONCLUSIONS

In conclusion, the concomitant intake of Molokhia soup with ciprofloxacin can significantly reduce drug bioavailability. In this

study, Molokhia soup caused a delay of the *in vivo* absorption by approximately 30 minutes in healthy rabbits. Moreover, there was a reduction in C_{max} by about 30%. Food viscosity appears to be an important parameter affecting drugs absorption.

This kind of undesirable food effect may lead to sub-therapeutic levels, treatment failure and bacterial resistance. Therefore, care should be given to avoid administering Ciprofloxacin with Molokhia soup and patients should be informed about such food-drug interactions. Further studies are needed to evaluate the effect of Palestinian food on the bioavailability of the different drugs.

CONFLICT OF INTERESTS

The authors report no conflicts of interest in this manuscript.

REFERENCES

1) Fleisher D, Li C, Zhou Y, Pao L-H, Karim A. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clin Pharmacokinet. 1999; 36(3): 233-54.

2) Abrahamsson B, Albery T, Eriksson A, Gustafsson I, Sjöberg M. Food effects on tablet disintegration. Eur J Pharm Sci. 2004; 22 (2): 165-72.

3) Radwan A, Amidon GL, Langguth P. Mechanistic investigation of food effect on disintegration and dissolution of BCS class III compound solid formulations: the importance of viscosity. Biopharm Drug Dispos. 2012; 33(7): 403-16.

4) Anwar S, Fell J, Dickinson P. An investigation of the disintegration of tablets in biorelevant media. Int J Pharm. 2005; 290(1): 121-7.

5) Parojčić J, Vasiljević D, Ibrić S, Djurić Z. Tablet disintegration and drug dissolution in viscous media: Paracetamol IR tablets. Int J Pharm. 2008; 355(1): 93-9.

6) Kalantzi L, Polentarutti B, Albery T, Laitmer D, Abrahamsson B, Dressman J, et al. The delayed dissolution of paracetamol products in the canine fed stomach can be predicted in vitro but it does not affect the onset of plasma levels. Int J Pharm. 2005; 296(1): 87-93.

7) Reppas C, Eleftheriou G, Macheras P, Symillides M, Dressman J. Effect of elevated viscosity in the upper gastrointestinal tract on drug absorption in dogs. Eur J Pharm Sci. 1998; 6(2): 131-9.

8) Hooper D, Wolfson J, Ng E, Swartz M. Mechanisms of action of and resistance to ciprofloxacin. Am J Med. 1987; 82(4A): 12-20.

9) Lindenberg M, Kopp S, Dressman JB. Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. Eur J Pharm Biopharm. 2004; 58(2): 265-78.

10) Breda SA, Jimenez-Kairuz AF, Manzo RH, Olivera ME. Solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin pharmaceutical derivatives. Int J Pharm. 2009; 371(1): 106-13.

11) Kyriacos SB, Boukarim C, Safi W, Mroueh M, Maroun AB, El-Khoury G, et al. In vitro testing of ciprofloxacin formulations and preliminary study on BCS biowaiver. J Food Drug Anal. 2009;17(2):78-84.

12) Lockley M, Wise R, Dent J. The pharmacokinetics and tissue penetration of ofloxacin. J Antimicrob Chemother. 1984; 14(6): 647-52.

13) Fahmy S, Abu-Gharbieh E. In vitro dissolution and in vivo bioavailability of six brands of ciprofloxacin tablets administered in rabbits and their pharmacokinetic modeling. Biomed Res Int. 2014; 590848 (1): 1-8.

14) Al-Ghazawi M, Aburjai T, Shraim N, Bani-Jaber A, AbuRuz S. Effect of Licorice Extract on the Pharmacokinetics of Ciprofloxacin in Rabbits after Oral Administration Using an Improved High-performance Liquid Chromatography Assay. JJPS. 2012; 5(2): 120-30.

15) Ledergerber B, Bettex J-D, Joos B, Flepp M, Lüthy R. Effect of standard breakfast on drug absorption and multiple-dose pharmacokinetics of ciprofloxacin. Antimicrob Agents Chemother. 1985; 27(3): 350-2.

16) Neuvonen PJ, Kivistö KT, Lehto P. Interference of dairy products with the absorption of ciprofloxacin. Clin Pharmacol Ther. 1991; 50(5-1): 498-502.

17) Turnidge J. Pharmacokinetics and pharmacodynamics of fluoroquinolones. Drugs. 1999; 58(2): 29-36.

18) Idirs S, Yisa J, Ndamitso M. Nutritional composition of Corchorus olitorius leaves. Anim Prod Res Adv. 2009; 5(2): 83-87.

19) Yamazaki E, Kurita O, Matsumura Y. Hydrocolloid from leaves of Corchorus olitorius and its synergistic effect on κ-carrageenan gel strength. Food hydrocoll. 2008;22(5):819-25.

20) Kordick DL, Papich MG, Breitschwerdt EB. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats. Antimicrob Agents Chemother. 1997; 41(11): 2448-55.