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Original Research Article

Effect of Carica papaya (Linn) aqueous leaf extract on pharmacokinetic profile of ciprofloxacin in rabbits

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Abstract

Purpose: *To investigate the fate of ciprofloxacin, after concomitant administration with the aqueous leaf extract of Carica papaya, which herbal practitioners in Nigeria have found helpful in the treatment of painful crisis in sickle cell anaemia (SCA) patients.*
Method: *Thirteen rabbits were fasted for 12 h and given by oral route 20 mg/kg ciprofloxacin (control group); after a 3-week crossover period, the animals were given 500 mg/kg aqueous extract of Carica papaya followed by 20 mg/kg ciprofloxacin (treatment group). Blood samples were collected over a period of 0 - 24 h post-dosing, and pharmacokinetic profile of ciprofloxacin in plasma sample determined using a validated high performance liquid chromatography (HPLC) method.*
Results: *Time to attain maximum plasma ciprofloxacin concentration (T_{max}), lag time and clearance (CL/F) were higher in the treated group; all other pharmacokinetic parameters showed significant decrease in the treated group ($p < 0.005$) compared to the control group. The slow rate of elimination of ciprofloxacin in the control group ($0.32 \pm 0.11\ h^{-1}$) compared to the treated group ($0.21 \pm 0.07\ h^{-1}$) was not due to kidney impairment as plasma creatinine level indicated that kidney function was within normal range (0.68 ± 2.78 mg/dl).*
Conclusion: *The results of the study show that there is interaction between ciprofloxacin and Carica papaya. This interaction can be avoided by taking ciprofloxacin at least 3 h prior to administration of the leaf extract of C. papaya.*

Keywords: *Carica papaya, Ciprofloxacin, Sickle cell anaemia, Herb-drug interaction, Pharmacokinetics*

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INTRODUCTION

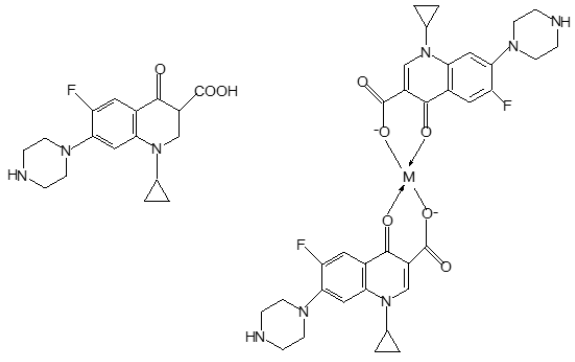
The use of herbal preparations for medicinal purposes is in parallel with conventional drugs in some populations. Also, fortification or concurrent administration of conventional drugs with herbal preparation is on the increase. It is known that herbs can affect body functions; therefore when taken concurrently with conventional drugs, interactions are possible. Thus a great concern has arisen on possible drug-herb interaction.

Ciprofloxacin is a fluoroquinolone antibiotic with excellent activity against gram positive and gram negative bacteria as well as Mycobacteria. Oral absorption of this drug has been reported to be affected by its metal cation complexation (Figure 1) which has been extensively studied for antacids [1-3] mineral supplement [4] and milk products [5,6]. However, literature on its

interaction with ciprofloxacin is lacking. Ciprofloxacin is well prescribed for the treatment of infections associated with sickle cell anaemia (SCA): a hereditary blood disorder caused by a single amino acid substitution [Glu→Val] at the sixth position of the β-chain haemoglobin. Similarly, herbal preparation of the leaf of *Carica papaya* is in good use, traditionally for the relief of painful crisis associated with SCA in Nigeria.

Carica papaya L., (Caricaceae) which is widely cultivated for its edible melon-like fruit is available throughout the year [7]. Phytochemical studies show *C. papaya* to contain vitamin C, vitamin E, pectin, carotinoids, alkaloids, carpain, nicotine, flavonols, tannins, terpinenes, enzymes papain and chymopapain [8,9]. These enzymes are similar to pepsin and help to digest protein in the body, used to relief indigestion, dyspepsia, reducing enlarged tonsils [9]. Flowers of *C. papaya* are used in the treatment of jaundice [personal communication] and inner bark used for sore throat [10]. The extract of unripe fruit and dried leaves of *C. papaya* have been reported to possess antisickling properties, thus used as an antisickling agent in the management of SCA in Western Nigeria [10,11].

The frequent use of ciprofloxacin and *C. papaya* in the management of SCA in Western Nigeria with no literature on their possible interaction prompted our study. We therefore investigated any possible interaction between these agents.



A B

Figure 1: Structure of ciprofloxacin (A) and ciprofloxacin-metal chelate complex (B).

EXPERIMENTAL

Plant material

The leaves of *Carica papaya* were collected from a farm in Lagos, Nigeria in August, 2012 and identified by Mr. Usang Felix of the Forestry Research Institute of Nigeria (FRIN) where a voucher specimen (FHI: 106994) has been deposited. The leaves were dried at room temperature and ground into powder. The powdered leaf, 500 g was extracted by exhaustive Soxhlet extraction into water for 24 h (pH 6.4). The aqueous crude extract was freeze dried to obtain a weight of 237.15 g (47.43 %w/w). The crude extract residue was stored at 4 ^{o}C until used.

Animals

Thirteen rabbits weighing between 1.8 and 2.2 kg were obtained from an open market and housed in a well-ventilated Laboratory Animal Centre of the College of Medicine of the University of Lagos, Nigeria under standard laboratory condition (12:12 h dark/light cycle). This study was approved by the Health Research and Ethics Committee (HREC) of the College of Medicine of the University of Lagos, Nigeria (CM/HREC/PHM/09/16/014).

The animals were cared for and used in accordance with the Institute of Laboratory Animal Research (ILAR) guidelines for care and use of animals in experimental studies [12]. The animals were allowed to acclimatize for 21 days during which they had free access to commercial pellet diet (Pfizer Feeds, Ibadan, Nigeria, Plc.) and water *ad libitium*.

Chemical reagents

All reagents were of analytical grade from Sigma Chemical Co. (St Louis, MO) unless otherwise stated. Ciprofloxacin hydrochloride reference standard was a gift from Swiss pharma Nigeria. Ciprofloxacin tablets (Ciprogem®) were purchase from a pharmaceutical shop in Lagos metropolis. De-ionized distilled water was used throughout the study. Tinidazole, potassium dihydrogen phosphate, tetrabutylammonium hydroxide, nitric acid, hydrochloric acid were from the British Drug House (BDH), Poole, England.

HPLC instrumentation and chromatographic conditions

The HPLC System was (Agilent 1100 series pump (Serial No DE 43630403, Product No G1311A, Hewlett Packard, Germany) equipped with Rheodyne 7725i injector (USA) coupled to UV detector (Serial No JP43826101 product No G1314A, Japan). The degasser (Serial No JP40720373, Product No G1379A, Japan) was used to remove possible gas in the mobile phase. The chromatographic responses were recorded by Agilent ChemStation Software (Agilent technologies, USA) running on Compaq

compatible personal computer (Hewlett Packard) with an Inlet Pentium processor operating at 2799 MHz under Microsoft window operating environment and a pin writer hp DeskJet 5652 printer. The chromatographic separation was performed at column temperature of 25 ^{o} C on an inert silica C_{18} zorbax SB column, 5 µm particle size, 250 mm x 4.6 mm i. d. protected by a Lichrospher Si 60 guard column 30 mm x 4.6 mm i.d., which was placed between the injector and the analytical column. The pH meter was Thermo Orion, model 420A (serial No. 071838, Beverly, MA, USA).

The mobile phase consisted of pH 3.5 phosphate buffer (2 mM): acetonitrile (60:40 v/v) distributed by the gradient pump at a flow rate of 1.0 ml/min and the effluent monitored by the ultraviolet detector at 278 nm. The phosphate buffer was prepared fresh each day of the experiment and consisted of potassium dihydrogen phosphate, tetrabutylammonium hydroxide and phosphoric acid in water.

Atomic absorption spectrophotometer operating conditions

The composition of metals in the leaves of *C. papaya* were analyzed using atomic absorption spectrophotometer (Buck Scientific, Model 210VGP AAS, USA) equipped with deuterium background corrector and air-acetylene flame atomizer. The instrument was optimized by altering the wavelength, slit width, lamp current and sample energy for each metal to give maximum signal strength (Table 1). The spectrophotometer was calibrated using 100 and 1000 µg/l of zinc working standards prepared in de-ionized water. Calibration curves of absorbance values versus concentration for each metal were constructed from different concentration (0 to 3000 µg/l) of freshly prepared specific metal in de-ionized water. Except for Na and K that were determined by emission mode, all other metals were determined by absorption mode.

Measurement of mineral cation content of *Carica papaya*

The apparatus used for this study were thoroughly washed and rinsed with de-ionized water. They were thereafter soaked in 10 % HNO_3 for 24 h and again rinsed severally with de-ionized water and kept to dry in a dust free room. The determination of the metals in the leaves of *C. papaya* followed a standard procedure as described by [13]. Briefly, 2 g of powered leaves of *C. papaya* was sieved and subjected to dry ash in a clean porcelain crucible in a muffle furnace at 550 °C. The resultant dull grey ash was dissolved in 5 ml of HNO_3/HCl/H_2O (1:2:3 v/v/v) and heated gently until brown fumes disappeared. To the ash mixture was added 5 ml of de-ionized water; heated and the colourless solution which was filtered into a 100 ml flask using ashless Whatman filter paper was used for elemental analysis. The concentration of each element in the sample was estimated using their respective standard curve and calculated as mg metal per 100 g of the plant material.

Drug dosing and sample collection

The rabbits were fasted for 12 h prior to the study. On the day of the study, each animal was given 20 mg/kg body weight of ciprofloxacin (Ciprogem®, Gemini Pharmaceutical, Lagos, Nigeria) by gastric probe. Whole blood, 5 ml, was withdrawn from the retro orbital plexus of each animal before (0 hr) and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post dosing into heparinized bottles and centrifuged immediately at 3000 g for 15 min.

Table 1: Instrumental operating conditions for the determination of metals in *Carica Papaya*

Metal ions	Operating conditions				
	Wave length (nm)	***Slit width (nm)***	***Lamp current (mA)***	***Sample energy (ev)***	***Instrumental detection limit (mg/l)***
Ca	422.7	0.7	4.0	3.912	0.010
Cu	324.7	0.7	2.0	3.938	0.005
Cr	357.9	0.7	2.5	3.567	0.050
Fe	248.3	0.2	15	3.436	0.050
K	766.5	0.7	2.0	-	0.010
Mg	285.2	0.7	2.5	3.717	0.003
Mn	279.5	0.2	3.0	3.937	0.050
Na	589.0	0.2	5.0	-	0.050
Pb	283.3	0.7	3.5	2.874	0.030
Zn	213.9	0.7	3.0	3.237	0.005

The plasma was carefully collected using Pasteur pipette and stored frozen at -80 °C until assayed. In another study, after a three week washout period, the rabbits were dosed orally with aqueous leaf extract of *Carica papaya*, 500 mg/kg body weight followed 30 min after with ciprofloxacin, 20 mg/kg body weight. Blood samples were withdrawn and treated as in the first procedure. Plasma creatinine was also estimated by the method of Perrone *et al* [14] to eliminate possible interference of kidney function on the results of our study.

Validation studies

Calibration curve

Drug-free plasma was spiked with ciprofloxacin standard solution to obtain 2, 4, 6, 8 and 10 μg/ml with each concentration containing 10 μg/ml tinidazole as the internal standard. The peak area ratio of ciprofloxacin to tinidazole, was plotted against the corresponding concentration of ciprofloxacin and a linear regression calculated. The calibration study was conducted in 10 replicates.

Precision and accuracy

Method validation was performed using the frozen drug-free plasma sample with ciprofloxacin, 2 to 10 μg/ml. Accuracy, assessed by recovery efficiency (n=10) was determined by comparing the peak area ratio of the spiked plasma with the peak area ratio obtained by direct injection of the standard solutions of the same concentration.

The intra and inter day assays for precision and accuracy were obtained in 10 replicate assays at varying concentrations of 2 to 10 μg/ml. The relative standard deviation (RSD) was used for the assessment of accuracy and precision. Working standard solutions were made fresh each day of the assay.

Assay of ciprofloxacin

The concentration of ciprofloxacin in plasma was determined in 10 replicates by a validated HPLC method [15], with slight modifications. One hundred μl of tinidazole (IS, 10 μg/ml) was added to 0.5 ml plasma followed by acetonitrile, 1 ml, to precipitate protein. The mixture was centrifuged at 3,000 g for 15 min and 20 μl of the supernatant was injected into the HPLC system for chromatographic separation. The concentration of ciprofloxacin was estimated from the calibration curve using calibration equation of $y = mx + C$.

Pharmacokinetic analysis of data

The plasma concentration time data was obtained by a one compartmental analysis. The systemic exposure (area under the curve; AUC) was estimated by the trapezoidal rule method for observed values and this was extrapolated to infinity ($AUC_{0\rightarrow\infty}$). The pharmacokinetic parameters, which comprised of time to peak plasma concentration (T_{max}), peak plasma concentration (C_{max}), volume of distribution (V_d), elimination half life ($t_{1/2}$), clearance (CL) were estimated with standard formulae [16].

Statistical analysis

Data are expressed as mean ± SD (standard deviation). Statistical difference among groups was assessed by the unpaired Student t- test with the level of statistical significance set as $p < 0.05$

RESULTS

Assessment of metal ion content in *Carica papaya*

The determination of the metal content in the leaves of *Carica papaya* (Table 2) revealed the presence of Ca, Cu, Fe, K, Mg, Mn, Na and Zn. The toxic heavy metals - lead and chromium were below the detection limit. The low levels of the toxic heavy metals might be an evident of lack of soil contamination of the plantation sites of the *Carica papaya*.

Table 2: Mean content of metals in *Carica papaya* leaf extract

Mineral	Content
Ca	1134.50 ± 17.93
Cu	4.51 ± 10.71
Fe	363.74 ± 25.09
K	1404.70 ± 11.09
Mg	401.67 ± 10.41
Mn	5.73 ± 12.63
Na	142.87 ± 35.17
Zn	5.27 ± 17.03
Pb	Not detected
Cr	Not detected

Values are mean ± SD in mg/100 g (n = 5)

Validation data

The calibration curve was linear with a correlation coefficient, r^2 of 0.9997 and a regression equation of $Y = 0.0170X + 0.0019$ (X = concentration of ciprofloxacin and Y = peak area ratio of ciprofloxacin. For the intra or inter day assays (n = 10 each), RSD were less than 5 % in each concentration (Table 3), this shows

precision to be good within the range of concentrations studied. Accuracy expressed as percentage bias was – 4.32 or – 5.25 % for the intra and inter day assayed (10 µg/ml; n = 10). Accuracy was also assessed from the percent recovery of ciprofloxacin that had been added to plasma. Mean recovery for intra-day assay was 97.15 ± 0.15 and 99.77 ± 0.57 % for the 2 and 10 µg/ml, respectively. The ciprofloxacin solutions in plasma stored frozen at – 20 ^{o}C was stable over the period of experimental analysis. Repeated freezing and thawing of the plasma had no effect on the concentration of ciprofloxacin.

Pharmacokinetics of ciprofloxacin: effect of *Carica papaya* co-administration

The mean plasma concentration-time curves of the oral administration of ciprofloxacin, 20 mg/kg body weight in the control group and the group with concomitant administration of *C. papaya*, 500 mg/kg body weight are shown in (Figure 2). The pharmacokinetic parameters are summarized in (Table 4); absorption of ciprofloxacin was more rapid in the control group than in the group that consumed *C. Papaya*, which was evident by ciprofloxacin appearing in the plasma of the control group 10 min earlier than in the test group. C_{max} was significantly higher ($p < 0.005$) in the control group (3.45 ± 0.34 mg/L) compared to the treated group (1.71 ± 0.41 mg/L) indicating a 50 % reduction in its maximum concentration. The corresponding T_{max} was found to be longer by 13 % in the treated group than in the control with values of 2.02 ± 0.45 and 1.79 ± 0.33 h respectively. C_{max} was followed by a concentration decay that could be fitted to a straight line on semi logarithmic scale, indicating first order mode of elimination. Elimination half-life ($t_{½el}$) was 3.25 ± 0.31 and 2.20 ± 0.11 h for control and treated groups respectively being slower by 32 % in the treated group ($p < 0.05$). The significant high plasma concentration of ciprofloxacin resulted in significant ($p < 0.05$) increase in AUC in the control group compared to the treated. There apparent volume of distribution (V_d/F) was higher in the control group compared to the treated. Total clearance (CL/F) was found to be 0.13 ± 0.06 L/h/kg and 0.83 ± 0.10 L/h/kg for control and treated groups respectively.

Table 3: Assay precision and accuracy of ciprofloxacin determination in plasma

Nominal conc. (µg/ml)	Found conc. (µg/ml)	RSD[a] (%)	Accuracy[b] (%)	Recovery (%)
Intra-day				
2	1.94 ± 0.07	3.61	-3.10	97.15 ± 0.15
4	3.83 ± 0.14	3.66	-4.32	95.75 ± 0.57
6	5.88 ± 0.23	3.91	-2.31	98.93 ± 0.45
8	7.79 ± 0.31	3.98	-2.78	97.74 ± 0.72
10	9.92 ± 0.45	4.51	-0.83	99.77 ± 0.57
Inter-day				
2	1.98 ± 0.09	4.55	-1.00	99.37 ± 0.33
4	3.79 ± 0.11	2.90	-5.25	94.87 ± 0.17
6	5.93 ± 0.24	4.05	-1.17	98.52 ± 0.32
8	7.94 ± 0.19	2.39	-0.75	99.42 ± 0.24
10	9.86 ± 0.23	2.33	-1.40	98.68 ± 0.61

Values are expressed as mean ± SD (n = 10); [a] % RSD = SD/mean x 100; [b] % accuracy = {(found conc. – nominal conc.)/nominal conc.}100

Table 4: Pharmacokinetic parameters of ciprofloxacin after a single 20 mg/ke oral dose with or without aqueous extract of *Carica papaya*

Pharmacokinetic parameter	Mean ± SD		
	Ciprofloxacin	Ciprofloxacin + *C. papaya*	*P*-value
Absorption			
C_{max} (mg/L)	3.45 ± 0.34	1.71 ± 0.41**	< 0.05.
T_{max} (h)	1.79 ± 0.24	2.02 ± 0.39	
Lag time (h)	0.25	0.45	
Elimination			
$T_{½el}$ (h)	3.25 ± 0.31	2.20 ± 0.31*	< 0.005
K_{el} (h^{-1})	0.32 ± 0.11	0.21 ± 0.07	
F	0.8614	0.3275	
$AUC_{0-\infty}$ (mg h/L)	23.6 ± 1.73	11.8 ± 2.21	
V_d/F (L/kg)	2.41 ± 0.11	1.26 ± 0.20	
CL/F (L/h/kg)	0.13 ± 0.06	0.83 ± 0.104	

Values are mean ± SD (n = 7); * $p < 0.005$; ** $p < 0.05$

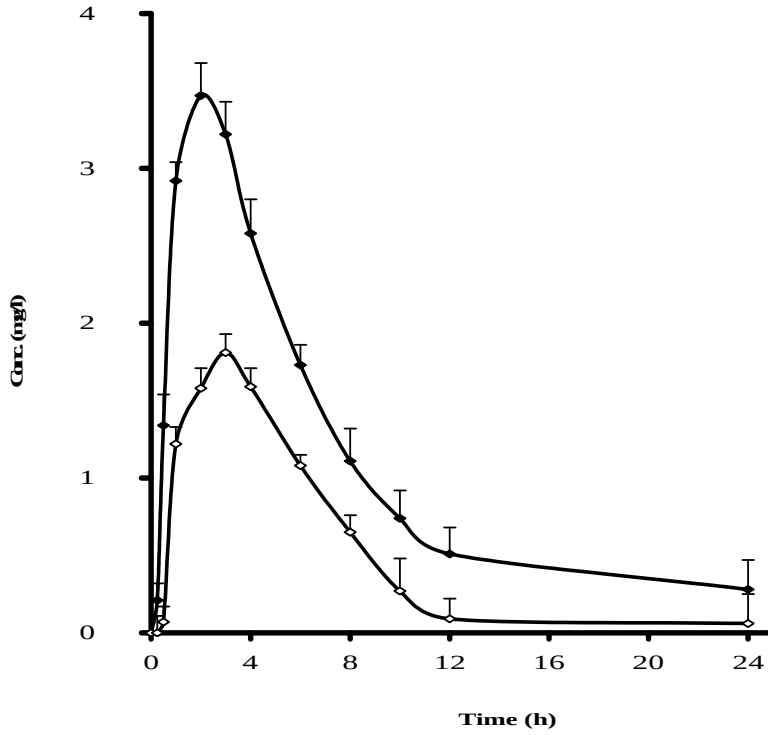


Figure 2: Mean plasma concentration-time curve of the oral administration of ciprofloxacin (20 mg/kg) without - ● - or with - ○ - co-administration of aqueous leaf extract of *Carica papaya* (100 mg/kg)

DISCUSSION

In recent times, high consumption of herbal remedies has increased the possibility of interaction between conventional drugs and natural products. The mixing of herbal remedies and prescription drugs could be harmful to health, they can alter the way the body metabolizes drugs, cancel the effect of drugs, increase or decrease the amount of drug in the blood stream or its impact to the body. An increase in a drug dosage could occur when an herb component enhances absorption of a drug, or inhibits the enzymes that break down drugs in preparation for elimination. Similarly, a decrease in the amount of drug could occur by herb components binding up the drug and preventing it from getting into the blood stream or by stimulating the production and activity of enzymes that degrades the drugs.

Our study investigated for the first time the possible interaction of *Carica papaya* on the absorption and pharmacokinetics of ciprofloxacin after concomitant administration. Ciprofloxacin is usually well absorbed but in the presence of agents containing some cations, its absorption is impaired [4,17,18]. The leaf of *C. Papaya*, as demonstrated in this study, contains some cations like Mg, Ca, Na, K, Fe, Cu, Zn and Mn (Table 2); and is line with the work of [19-21]; with this, the result of our pharmacokinetic study was not unexpected. When ciprofloxacin was co-administered with *C. papaya*, the peak plasma concentration (C_{max}) significantly decreased by 50 % with an increase in time to achieve the peak plasma concentration, T_{max} ($p \leq 0.05$, Figure 2). Similar result was obtained from the study of [4] with co-administration of norfloxacin, ofloxacin and ciprofloxacin with 100 mg of elemental iron. The faster a drug is absorbed, the greater the peak plasma concentration and shorter the time to peak plasma concentration [22]. It may therefore imply from our study, that the reduction in C_{max} and longer T_{max} may have been as a result of reduced rate of absorption in the presence of *C. papaya* which is be supported by the 3 fold increased in lag time observed in this study.

The elimination half-life of ciprofloxacin following co-administration with *C. papaya* was lower than the control. It is known that a drug with a short elimination half-life will be eliminated from the body much more quickly than a drug with longer half-life [22], the results of our study is therefore consistent with increased drug elimination, which may be evident by large drug clearance and reduced apparent volume of distribution in the treated group. The slow rate of elimination of

ciprofloxacin in the control group (0.32 ± 0.11 h^{-1}) compared to the treated (0.21 ± 0.07 h^{-1}) was not due to kidney impairment as the plasma creatinine level that indicate kidney function was within normal range (0.68 ± 2.78 mg/dl). There was reduction in the bioavailability of ciprofloxacin in the treated group compared to the control ($p < 0.05$). Reduced bioavailability in our study is supported by reduced rate of absorption and increased rate of elimination. The volume of distribution, Vd was reduced 2-fold with co-administration of *C. papaya*, implying reduced distribution of ciprofloxacin in this group of animals. This may be attributed to reduced rate of absorption or low lipophilicity leading to poor membrane permeability through the gut wall.

There are convincing reports that the impairment of the absorption of ciprofloxacin by metal cations is as a result of the formation of a water soluble, non-absorbable metal chelate complex with the 3-carboxyl and 4 keto oxygen groups of the ciprofloxacin molecule which are in close proximity [23,24] thus resulting in very low lipophilicity leading to poor permeation through the gut wall [1,6,17]. The presence of cations in the leaves of *C. papaya* may be sufficient in impairing the absorption of ciprofloxacin. Each cation in the leaf extract may result in the formation of ciprofloxacin-metal chelate complex, thus increasing the molecular size and bulkiness of the drug or form a chemical reaction that can lead to poor drug permeation through the gut wall. It is also presumed from the study that the formation of a ciprofloxacin-metal chelate complex is probably the cause of the reduction in ciprofloxacin bioavailability which occurred in the treated group.

CONCLUSION

Taken together, the findings of this study show that there is interaction between the extract of *C. papaya* and ciprofloxacin based on pharmacokinetic data. This interaction can be avoided by taking the ciprofloxacin at least 3 h prior to the administration of the leaf extract of *C. papaya*.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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