Original Article

Pharmacokinetics of Fluconazole tablets administered to healthy subjects

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ABSTRACT

Fluconazole is an antifungal agent used for treating infections caused by different sorts of fungus and yeasts. Despite the wide usage of fluconazole, up to date, no information is available in the literature concerning the pharmacokinetic characteristics of fluconazole tablets administered to the Arabic population. Since the therapeutic doses of the drug range from 50-400 mg, therefore, the pharmacokinetics of fluconazole 200 mg tablet was investigated in 35 Arabic adult healthy fasting men using plasma concentrations obtained from each one. After overnight fasting of 12 hours, a single dose of the drug was administered and blood samples were withdrawn from each individual before drug intake, and then 17 serial blood samples were obtained for up to 120 hours after drug intake. The current research introduced the primary and the secondary pharmacokinetic parameters of fluconazole 200 mg tablets including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $K_{elimination}$ (λ_z), T_{half}, MRT, Cl, and Vd which were measured by non-compartmental analysis of the concentrations versus time data of each individual. Moreover, it was found that fluconazole 200 mg tablet was well tolerated by all individuals and no incidence of serious adverse effects was observed.

Keywords: Fluconazole, Tablet, Pharmacokinetics, Healthy subjects

Introduction

Fungal infections have become a serious concern worldwide [1]. Fluconazole is used for preventing and treating various and many kinds of systemic and superficial yeast and fungal infections in adults and pediatrics [2-4]. The drug is available in different dosage forms and routes of administration including oral tablets (50, 100,150, 200 mg), oral capsules (150 mg), powder for an oral suspension containing 10 and 20 mg fluconazole per ml, and intravenous injection containing 2 mg of the drug per ml [3, 4]. Very recently, other new dosage forms using advanced pharmaceutical technologies were tried such as micro-emulsification [5-7], nano-emulgel formation [8], and oral strip [9].

Fluconazole is indicated for vaginal candidiasis, oropharyngeal and oesophageal candidiasis. The drug is also effective in cases including cryptococcal infections in patients with Acquired Immunodeficiency Syndrome (AIDS), meningitis, candida urinary tract infections, peritonitis, and systemic candida infections (candidemia, disseminated candidiasis, and pneumonia). Besides, fluconazole is described for prophylaxis to reduce the incidence of candidiasis for patients undergoing bone marrow transplantation who are using radiation therapy and/or cytotoxic chemotherapy [3, 4]. Fluconazole was also prescribed recently for patients with diabetes mellitus [10].

Interestingly, fluconazole possesses unique and distinguished pharmacokinetic characteristics [3, 4, 11] in comparison to

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Ammoo AM, Al-Tamimi DJJ, Al-Mahroos MIA, Al-Tamimi MJJ, Ibraheem JJ. Pharmacokinetics of Fluconazole tablets administered to healthy subjects. J Adv Pharm Educ Res. 2021;11(2):92-9. https://doi.org/10.51847/UR4Sj4hR0i

many other widely used drugs such as azithromycin [12], betahistine [13], doxazocin [14], cefixime [15], cyclosporine [16], amlodipine, valsartan, and hydrochlorothiazide [17]. There is a close similarity in the fluconazole's pharmacokinetics after intravenous, oral tablets, and oral suspension. Oral intake of fluconazole shows rapid and nearly complete absorption from the gastrointestinal tract, and the absolute bioavailability was found to be above 90% which indicates that almost all the orally-administered fluconazole escape first-pass effect (including metabolism in the liver) and reaches the systemic circulation. Thus, intravenous, oral tablets and oral suspension of fluconazole are given in identical daily doses, and consequently, dose adjustment is not necessary for clinical practice if there is a need for changing from one dosage form to another, i.e., oral tablets, to oral suspension or intravenous route, or vice versa. In addition to the above-mentioned data, fluconazole follows linear/dose-proportional pharmacokinetics in terms of its primary pharmacokinetic parameters including maximum plasma concentration, the area under plasma concentration-time curve, and elimination from the body. Furthermore, food intake possesses no significant impact on the absorption of fluconazole from gastrointestinal tract and its bioavailability [3, 4, 11].

Oral fluconazole attains maximum plasma concentration within 1-3 hours. The average terminal plasma elimination half-life of the drug is about 30 hours (ranging from 20-50 hours). Besides, in clinical practice, the relatively long terminal plasma elimination half-life of fluconazole introduces the advantage and the basis for once-daily dosing. About 80% of administered fluconazole is excreted unchanged in the urine. The average apparent volume of distribution of fluconazole is almost identical to the volume of total body water which is about 50 liters, its mean total body clearance is nearly 1 liter per hour, and its degree for binding to plasma protein binding is low ranging from 11-12%. The extent of fluconazole binding with plasma proteins possesses no clinical importance. The drug distributes widely throughout body organs, tissues, and fluids involving sputum, saliva, and cerebrospinal fluids. The rapid and high absorption after oral administration of fluconazole, in addition to the relatively high apparent volume of distribution, and long terminal plasma elimination half-life and mean residence times, make the drug effective at a wide range of body sites [3, 4, 11].

Using antimicrobial agents for prevention and treatment of infections possesses special concern for successful therapy to get the necessary therapeutic plasma concertation with optimal effect and minimal adverse effect [12, 15, 18-20]. If the administered antimicrobial dose yield plasma levels below the minimum therapeutic levels and consequently it is not adequate to produce the required efficacious plasma levels which assure destruction of the microorganism which causes the infection, then this will lead to the failure of drug therapy and the infection will persist, and the potential risk for developing resistance to the drug may be escalated. On the other extreme, If the given dose of the antimicrobial agent yields plasma levels above the maximum therapeutic levels, consequently drug therapy will be associated with adverse effect(s) which lead to stopping the drug usage, and replacement by other antimicrobial, or using a combination of antimicrobial agents which have many negative sides in term of the health and cost to the patient [12, 15, 18-20].

Therefore, many pharmacokinetics, bioavailability, and bioequivalence researches were conducted in different countries and for almost all drugs to know the pharmacokinetic behaviors of the drug in each nation [12-18, 21-29]. Among the researches conducted for fluconazole in particular, were for Thai [21], Arabic [18, 22, 23], Brazilian [24], Spanish [25], Serbian [26], Mexican [27], Bangladeshi [28], and Chinese individuals [29].

Up to date, no published data is available regarding the pharmacokinetic behaviors of fluconazole 200 mg tablets in the Arabic nation. Thus, the purpose of this investigation was to introduce the pharmacokinetics of fluconazole 200 mg tablets in healthy male adult Arabic subjects under fasting state since the therapeutic doses of fluconazole range from 50-400 mg daily.

Materials and Methods

Study design

The current investigation was conducted following a protocol that was prepared by the principal investigator/study director and adhering with the ICH guidelines on good clinical practice [30] and the recent version of the Declaration of Helsinki [31]. Before study execution, the study protocol was approved by the clinical investigator and the Institutional Review Board (IRB). The protocol contains the informed consent forms of the participants, and all details of this study including the clinical phase, bioanalytical procedures, and all pharmacokinetic calculations and data analysis. The participants were thoroughly informed about all study details and any potential risks which may be associated with the study, and their rights to withdraw from participation at any time during the research, and the financial compensation. The clinical investigator signed each consent form, and each participant gave his signed and written informed consent in addition to two witnesses before screening processes. Two original versions of the written and signed informed consents were submitted, one version was delivered to the participant to know and preserve his rights, and the other version was archived in the study files.

Inclusion criteria

Arabic healthy adult men were selected to participate in the current project. The inclusion criteria for participation included the following parameters: 1) The individual should comply with the requirements of the protocol and willing to undergo pre- and post-study examinations, 2) Ages between 18-48 years, 3) Body Mass Index (BMI) range 18-30, 4) Non-smoker or light smoker (less than ten cigarettes per day), 5) No history of alcohol and drug abuse, 6) No participation in any sort of clinical trials such as pharmacokinetics, bioavailability, and

bioequivalence, 7) No recent surgical operation and/or donation of blood for at least eight weeks prior the study, and, 8) No acute infection within at least two weeks prior the study.

The individual was regarded healthy and eligible for participation in the study based on the following parameters: 1) Personal interview with the participant, 2) Complete physical examinations, 3) Complete clinical examinations including normal vital signs (blood pressure, temperature, and pulse), normal ECG, no medical history of diseases including cardiovascular, respiratory, renal, hepatic, gastrointestinal, epilepsy, bleeding, severe anemia, coagulation disorders and psychiatric problems, 4) Clinical laboratory tests included normal biochemistry and hematology, negative (HIV, hepatitis B, and C), negative drug abuse tests, negative alcohol abuse tests, and normal routine urine analysis, 5) No history of hypersensitivity, allergy, and/or contraindication to fluconazole and any related agents.

Clinical phase

All the participants attended the clinical site before about 16 hours of fluconazole administration. Vital signs, alcohol, and drug abuse examinations were achieved for each individual, and subsequently, each eligible individual was given a confidential code number to be used during the entire investigation and to preserve the privacy of the participant. Thereafter, standard dinners were served at 12 hours pre-dosing. All participants were confined at the clinical site to assure complete fasting after dinner intake (other than water) until drug administration. Moreover, all participants stayed in the clinical site until 24 hours post-dosing for blood samples collection, and they were asked to return to the clinical site for further blood sampling at 48, 72, 96 and at 120 hours post-dosing which is the time of last blood sample withdrawal, and discharge of the participants.

Fluconazole 200 mg tablet (Diflucan®, Pfizer) was administered to each individual with 240 ml water after overnight fasting of 12 hours. A mouth check was achieved after drug intake by the clinical staff to ensure drug intake by the participant. A standard lunch, snack, and dinner were served at 4, 8, and 12 hours of drug administration, respectively. No water was allowed 2 hours pre- and 2 hours post-dosing, then water was allowed as desired. The participants were not allowed to lie or sleep during the first hours of fluconazole intake and they were stayed upright sitting or standing.

Blood sampling

Venous blood samples were taken from each individual at zero time (about one-hour pre-dosing) as a baseline, and then at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96 and eventually at 120 hours post-dosing. A total of 18 blood samples were taken from each individual during the whole study. Immediately after each blood sample withdrawal, 1 ml of saline (containing 0.5 IU heparin) was injected to prevent clotting and avoiding closure of the cannula. In addition to that, before the next blood sample withdrawal, nearly 0.5 ml of blood was discarded from the cannula to get rid of any remained blood in the cannula. The time for actual blood sample withdrawal was registered. Each blood sample was directly transferred to a tube containing heparin and immediately centrifuged for 5 minutes at 4000 rpm for separation of plasma. The separated plasma samples were saved as two aliquots in a deep freezer at -20±10 °C until the day of measuring fluconazole's plasma concentrations. All the tubes used for blood and plasma samples collection were labeled according to a confidential in-house coding system solely known by the principal investigator and the quality assurance responsible. The code specifies the number of the protocol, subject confidential number, and the number of the blood/plasma sample obtained at each time point.

Safety evaluation

Fluconazole's tolerability and safety were evaluated by the clinical investigator and the clinical staff by observing, monitoring, and interviewing each individual for the presence of any potential Adverse Events (AEs), Adverse Drug Reactions (ADR), and Serious Adverse Effects (SAE). In addition to that, the vital signs including pulse, blood pressure, and temperature were monitored at approximately 1 hour before fluconazole administration, and then at 1, 2, 4, 6, 12, 24, 48, 72, 96, and at 120 hours after drug intake which is the time of discharge. Furthermore, clinical facilities were available to handle any urgent cases beyond the capability of the clinical site. The clinical investigator terminates the participation of any individual and during any time of the study if there are clinically significant changes in the vital signs, and the appearance of illness that continued participation may threaten and jeopardize the subject's health and well-being. Subject participation was also stopped in cases such as poor cooperation, bad compliance, and violation of any procedure specified in the study protocol.

Bioanalytical procedures

High-performance liquid chromatography coupled with UV detector was used for measuring fluconazole's plasma concentrations applying analytical methods described previously with phenacetin as the internal standard at 210 nm [32-36]. The analytical method was validated applying the current recommended FDA bioanalytical method validation guidelines [37-40]. The linearity of the standard calibration curve was constructed at concentrations ranges from 50-10000 ng/ml which is expected to cover fluconazole's plasma levels obtained after therapeutic oral doses of the drug as stated in previous literature concerning fluconazole's pharmacokinetics, bioavailability, and bioequivalence [11, 18, 20, 22, 24-29]. The lower limit of quantitation (LLOQ) of fluconazole in plasma was found to be 50 ng/ml.

As recommended by FDA guidelines [37-40], each analytical run contained all unknown authentic plasma samples collected from each subject containing fluconazole, together with the standard calibration curve, in addition to the low, medium, and high-quality control (QC) samples. Besides, fluconazole's plasma concentrations were not estimated by interpolation

and/or extrapolation below the LLOQ or above the upper limit of quantitation (ULOQ) of the constructed standard calibration curve with concentrations ranges from 50-10000 ng/ml plasma.

Pharmacokinetic analysis

Kinetica software was used for measuring all fluconazole primary and secondary pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $K_{elimination}$ (λ_z), T_{half}, MRT, Cl, and Vd applying standard methods [41-43]. Fluconazole's plasma concentrations versus time results were plotted by Excel software. The maximum plasma concentration (C_{max}), and the time to attain C_{max} (T_{max}) were obtained directly without interpolation from fluconazole's plasma concentration-time curve of each individual. Trapezoidal rule was applied for measuring the area under plasma concentration-time curve from the time of pre-dosing (t_0) and up to the time of last blood sample withdrawal (t_{last}) at 120 hours post-dosing (AUC_{0-t}). The extrapolated area under plasma concentration-time curve from t_{last} to infinity ($AUC_{t-\infty}$) was calculated from C_{last}/λ_z. The C_{last} is the last fluconazole's plasma level which meets or exceeds the LLOQ. The terminal elimination rate constant $K_{elimination}$ (λ_z) was determined applying the equation (log y=log a-bx) by least-square linear regression fitness of not less than 3 consecutive concentrations in the terminal phase of the log-concentration versus time curve of each individual. The terminal elimination half-life (T_{half}) was calculated as $0.693/\lambda_z$. The area under plasma concentration-time curve from t_0 to infinity ($AUC_{0-\infty}$) was measured from the sum of AUC_{0-t} + $AUC_{t-\infty}$. The % extrapolated AUC was estimated from the ratio of ($AUC_{t-\infty}/AUC_{0-\infty}$) ×100. The Mean Residence Time (MRT) was derived from AUMC/AUC. The AUMC is the area under the moment curve. The total body clearance (Cl) of fluconazole for each individual was measured as the dose of the drug given (200 mg) divided by the resulted $AUC_{0-\infty}$ of the individual, and his apparent volume of distribution (Vd) was calculated as the calculated Cl divided by the $K_{elimination}$ (λ_z) of the individual [41-43]. The mean±SD of fluconazole's plasma concentrations versus time data were graphed in rectilinear and semi-log plots.

Results and Discussion

Study conduct

Table 1 displays the demographic data and the baseline vital signs of all participants who completed the project. **Figure 1** shows the disposition of the individuals from the screening stage until the end of the study at the time of last blood sample withdrawal (120 hours post-dosing). Forty individuals were screened to account for any dropout and/or withdrawal of any subject (which is common in clinical trials). Three individuals were not eligible to be involved in this study according to the inclusion/exclusion criteria stated in the study protocol. Besides, 2 more individuals withdrew because of personal reasons. Thus, 35 eligible individuals were enrolled in the current project as depicted in **Figure 1**. Owing to the absence of dropout and withdrawal of any participants during the whole study, hence, all the 35 participants who started this project completed the study.

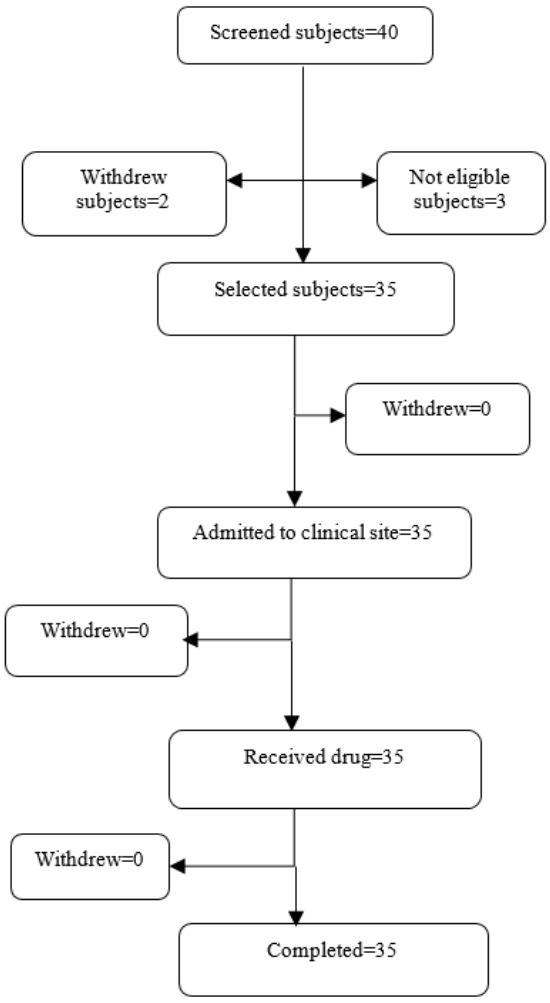


Figure 1. Flow Chart from Screening until Study Completion

Table 1. Demography and Base Line Vital Signs of 35 Individuals Completed the Study

characteristics	Mean±SD	(%CV)	Range
Age (years)	28.1±7.44	26.5	18-41
Height (m)	1.72±0.049	2.85	1.63-1.81
Bodyweight (kg)	71.3±8.97	12.6	60-87
BMI (kg/m^2)	24.04±2.43	10.2	20.3-28.1
Systolic blood pressure (mmHg)	117.9±4.0	3.4	110-125
Diastolic blood pressure (mmHg)	75.0±5.2	6.9	70-85
Pulse (beat per minute)	66.2±5.79	8.8	60-82
Temperature (°C)	36.9±0.14	0.038	36.7-37.1

Tolerability and safety

Fluconazole 200 mg tablets were well tolerated by all participants. Significant Adverse Events (AEs), Adverse Drug Reactions (ADR), and Serious Adverse Effects (SAE) which influence study execution and participant's safety were not recorded, and all participants were discharged without any

measurable changes in their baseline clinical data involving vital signs and clinical laboratory tests which were performed pre- and post-drug administration.

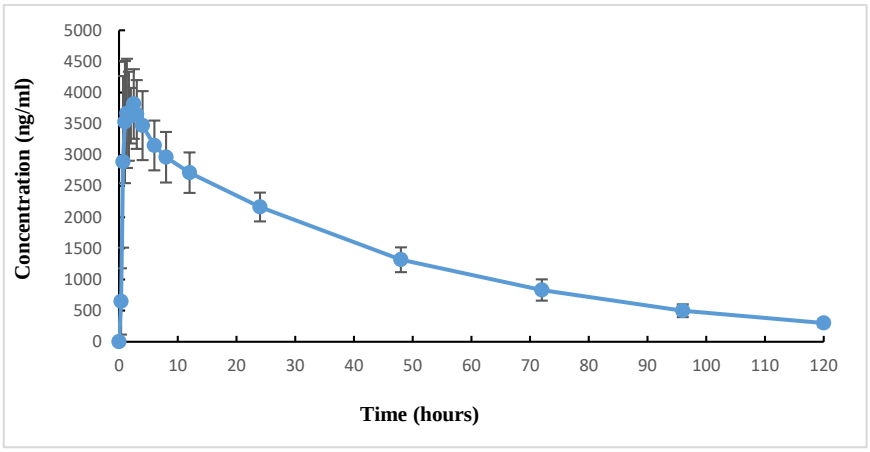
Bioanalytical data

The analytical method applied in this investigation [32-36] was successfully applied to demonstrate the pharmacokinetics after a single dose of 200 mg fluconazole tablets since all the validation parameters including accuracy, specificity, selectivity, sensitivity, and precision were well within the acceptance ranges required by the current recommended FDA bioanalytical method validation guidance [37-40]. Moreover, fluconazole's plasma concentration ranges from 50-10000 ng/ml applied were quite enough for covering the resulted plasma concentrations ranges as depicted in **Figure 2**.

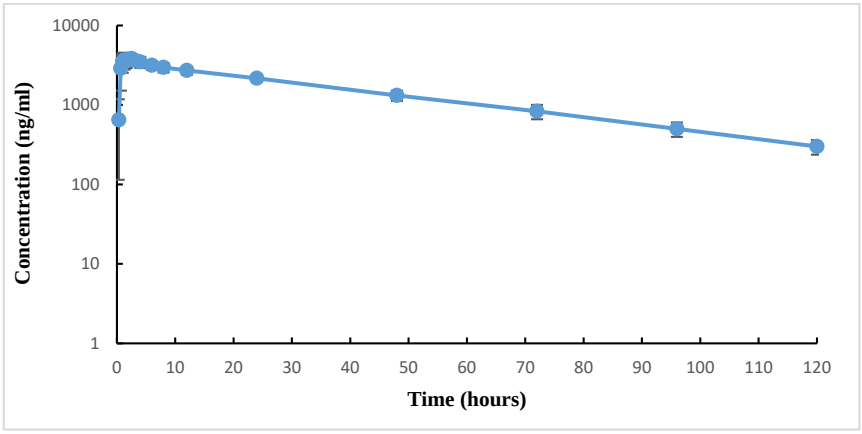
The intra- and the inter-batch precision and accuracy for fluconazole at low, mid, and high levels were below 8% which is less than FDA accepted ranges of 15-20% [37-40]. Fluconazole's recovery in all plasma concentration ranges were reproducible with mean value of 87±7 SD. The LLOQ of 50 ng/ml used in the current research was reproducible and identifiable. The chromatograms did not show any interfering peaks with fluconazole. The determination coefficients (r^2) were greater than 0.9991. Accordingly, the current bioanalytical method is appropriate enough for evaluating the pharmacokinetic, bioavailability, and bioequivalence of fluconazole [37-40, 42-44].

Plasma concentrations

Fluconazole's plasma concentrations versus time profiles (mean±SD) are depicted in rectilinear and semilog graphs as shown in **Figure 2**. Fluconazole is rapidly absorbed from tablets dosage form since the drug plasma levels were above the LLOQ of 50 ng/ml and in all the 35 investigational subjects. The drug was detected at the first blood sample withdrawal (i.e., 0.33 minutes post-dosing), and attained its maximum levels within 2 hours of drug administration, thereafter, fluconazole's plasma levels declined mono-exponentially with a relatively long terminal elimination phase **(Figure 2)**. The present findings are following many previously published pharmacokinetics, bioavailability, and bioequivalence studies [11, 18, 21, 22, 24-29].



a)



b)

Figure 2. Plasma Concentrations (Mean±SD) versus Time Profiles of Fluconazole after Administration of 200 mg Tablets, a) Rectilinear Graph, b) Semilog Graph

Pharmacokinetic data

Table 2 presents the descriptive statistics [44, 45] of all calculated fluconazole's pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $K_{elimination}$ (λ_z), T_{half}, MRT, Cl, and Vd. The average % extrapolated AUC was below 9% (range 5-14%) which indicates that blood sampling for 120 hours after

fluconazole dosing was very enough for the determination of fluconazole's plasma levels, and consequently reliable calculations of all drug pharmacokinetics was achieved **(Table 2)**. Interestingly, the average primary pharmacokinetic parameters Cl and Vd **(Table 2)** are similar to that reported in the literature [3, 4, 11]. Moreover, (after dose normalization), the mean pharmacokinetic parameters obtained after administering fluconazole 200 tablets to the Arabic population **(Table 2)** are almost comparable to the corresponding parameters obtained after administering fluconazole capsule or tablets to other populations including Arabic [18, 22], Serbian [26], Brazilian [24], Thai [21], Mexican [27], and Bangladeshi [28] as summarized in **Table 3**. Hence, it can be concluded from this investigation that ethnicity may not have a remarkable influence on fluconazole's pharmacokinetics **(Table 3)**.

Table 2. Pharmacokinetic Parameters of Fluconazole 200 mg Tablets

Statistics	C_{max} (µg/ml)	T_{max} (hr)	AUC_{0-t} (µg.hr/ml)	$AUC_{0-\infty}$ (µg.hr/ml)	% AUC_{extra}	λ_z (hr^{-1})	$T_{0.5}$ (hr)	MRT (hr)	Cl (ml/hr)	Vd (l)
Mean	4.3	1.87	157.0	172.0	8.6	0.022	34.6	47	1183	57.0
±SD	0.70	1.10	18.8	22.3	1.8	0.002	2.68	4.6	162	6.50
%CV	16.3	58.9	12.0	12.9	20.9	10	7.7	9.8	13.7	11.4
Min	3.2	0.67	122.0	129.0	5.4	0.018	27	39	961	47.0
Max	5.4	4.0	183.0	208.0	13.5	0.025	40	63	1551	70.3

Table 3. Average Pharmacokinetic Parameters of Fluconazole Obtained from Previous Studies in Comparison to the Current One After Administration of Various Dosage Forms and Doses to Different Populations.

Ref.	Year	Population	Dose (mg)	Dosage form	C_{max} (µg/ml)	$AUC_{0-\infty}$ (µg.hr/ml)	T_{max} (hr)	$T_{0.5}$ (hr)
[22]	2005	Arabic	150	Capsule	3.2	146.9	2.7	36.6
[26]	2005	Serbian	150	Capsule	2.6	107.0	2.4	35.1
[24]	2005	Brazilian	150	Capsule	3.6	153.3	3.0	30.0
[21]	2007	Thai	200	Capsule	4.0	156.5	2.5	30.9
[27]	2010	Mexican	150	Tablet	4.4	174.3	2.7	NA
[28]	2012	Bangladeshi	150	Capsule	2.5	94.4	2.2	44.6
[18]	2021	Arabic	150	Capsule	3.1	122.7	1.6	33.8
current	2021	Arabic	200	Tablet	4.3	172.0	1.9	34.6

Conclusion

The present investigation is the first one that demonstrated the pharmacokinetic characteristics of fluconazole 200 mg tablets administered to Arabic healthy men. All the primary and the secondary pharmacokinetic parameters of fluconazole were measured including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $K_{elimination}$ (λ_z), T_{half}, MRT, Cl, and Vd.

Acknowledgments: The authors greatly acknowledged the clinical team, the analytical staff, and the individuals who participated in the study. Thanks to Miss Manar Al-Tamimi for the technical assistance.

Conflict of interest: None

Financial support: None

Ethics statement: This study was carried out following the updated ICH guidelines for Good Clinical Practice (GCP), and provisions of the ethical principles for medical research involving human subjects stated in the latest version of the declaration of Helsinki.

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