# Bioequivalence and Pharmacokinetics Comparison of Two Formulations of Extended-Release Pentoxifylline Tablets in Healthy Subjects after Fasting and Fed Conditions

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## Abstract

The pharmacokinetics and bioequivalence of a newly developed extended-released (ER) tablet containing 400 mg pentoxifylline as a test product was compared with the reference brand product Trental® 400 mg ER tablet produced by Sanofi-Aventis. Two separate studies were conducted simultaneously. The first study was conducted under fasting condition, whereas, the second study was conducted under fed condition; using the same batches of the test and reference products in both studies. In each study, both products were administered to 32 healthy male adult volunteers applying a single-dose, two-treatment, two-period, two-sequence, randomized crossover design with one-week washout period between dosing. Twenty two blood samples were withdrawn from each volunteer over 24 hours period. Pentoxifylline concentrations were determined in plasma by a validated HPLC method according to FDA Bioanalytical Method Validation Guidance using UV detection and chloramphenicol as internal standard. The lower limit of quantitation of the drug in plasma was 5 ng/ml and the upper limit of quantitation was 500 ng/ml. From the plasma concentration-time data of each individual, the pharmacokinetic parameters; $C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, $T_{max}$, $\lambda_Z$ & $T_{0.5}$; were calculated applying non-compartmental analysis. Data of the test and reference products were statistically analyzed to test for bioequivalence of the two products, using criteria of FDA and EMEA Guidance. The pharmacokinetic parameters mentioned above were statistically analyzed by descriptive statistics, ANOVA test and 90% Confidence Interval (CI). ANOVA test involved the calculation of the effects of: treatment, period, sequence and subjects nested in sequence. According to the above guidance, the primary pharmacokinetic parameters used for bioequivalence testing, namely $C_{max,}$ $AUC_{0-t}$ and $AUC_{0-\infty}$ were also statistically analyzed by ANOVA & CI tests using the corresponding Ln-transformed values. The mean values $C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, $T_{max}$, $\lambda_Z$ & $T_{0.5}$ for the test formula obtained from the fasting study were; 144.4 ng/ml, 845.4 ng.hr/ml, 868.4 ng.hr/ml, 0.186 $hr^{-1}$, 3.29 hr, 0.561 $hr^{-1}$ and 1.65 hr, respectively; and the mean values of these parameters for the reference formula were; 150.0 ng/ml, 871.1 ng.hr/ml, 893.7 ng.hr/ml, 0.177 $hr^{-1}$, 3.70 hr, 0.558 $hr^{-1}$ and 1.59 hr, respectively. The mean values of the above mentioned parameters for the test formula obtained from the fed study were; 157.8 ng/ml, 826.5 ng.hr/ml, 853.8 ng.hr/ml, 0.198 $hr^{-1}$, 5.4 hr, 0.458 $hr^{-1}$ and 2.06 hr, respectively; and the mean values of these parameters for the reference formula were; 162.1 ng/ml, 869.7 ng.hr/ml, 894.8 ng.hr/ml, 0.196 $hr^{-1}$, 4.1 hr, 0.525 $hr^{-1}$ and 1.80 hr, respectively. Based on criteria of FDA and EMEA Guidance on Bioavailability and Bioequivalence, the results of the fasting and fed studies demonstrated bioequivalence of the two products under either condition. Accordingly, it is concluded that the newly developed ER tablet containing 400 mg pentoxifylline is bioequivalent to Trental® 400 mg ER tablet produced by Sanofi-Aventis.

**Keywords: Pentoxifylline, Pharmacokinetics, Bioequivalence.**

# مقارنة ألتكافؤ ألحيوى وحركية الدواء بين مستحضرين لحبوب البنتوكسيفلين طويلة ألمفعول (ER) فى أشخاص أصحاء مع الطعام وبدون طعام

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## الخلاصة

تمت مقارنة حركية الدواء والتكافؤ ألحيوى بين منتج دوائى جديد لحبوب ألبنتوكسيفلين طويلة ألمفعول (ER) تحوى على ٤٠٠ ملغ من عقار ألبنتوكسيفلين مع ألمنتج ألمرجعى ترنتال ٤٠٠ ملغ لشركة سنوفى أفينتس. تم عمل دراستين فى نفس الوقت. ألدراسة ألاولى تمت بدون طعام بينما انجزت ألدراسة ألثانية مع ألطعام باستعمال نفس ألوجبة من كل منتج. فى كل دراسه تم أعطاء كل منتج ألى ٣٢ متطوع باستعمال ألتصميم ألعشوائى وبفترة اسبوع بين ألجرع. تم سحب ٢٢ عينة دم من كل متطوع ولفترة ٢٤ ساعة وتم حساب تراكيز ألبنتوكسيفلين بالبلازما بواسطة HPLC / UV وحسب دستور ألتحليل ألآمريكى واستعمال عقار

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ألكرومفنيكول كمقياس داخلى. الحد ألادنى لتحليل الدواء فى ألدم 5 ng/ml والحد ألآعلى 500 ng/ml. معامل حركية ألدواء فى ألجسم وهى $C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, $T_{max}$, $\lambda_Z$ & $T_{0.5}$ تم حسابها ثم مقارنتها بين ألمنتج ألجنيس والمرجعى لغرض تقييم ألتكافؤ ألحيوى حسب الدستور ألأمريكى وألآوربى. بالنسبة للدراسة ألآولى بدون طعام فالنتائج لمعدل معامل حركية ألدواء $C_{max}$, $AUC_{0-t}$ , $AUC_{0-\infty}$ , $C_{max}/AUC_{0-\infty}$ , $T_{max}$, $\lambda_Z$ & $T_{0.5}$ وحسب ألتوالى للمنتج ألجنيس هى :
144.4 ng/ml, 845.4 ng.hr/ml, 868.4 ng.hr/ml, 0.186 $hr^{-1}$, 3.29 hr., 0.561 $hr^{-1}$ ,1.65 hr.
وللمنتج ألمرجعى هى:
150.0 ng/ml, 871.1 ng.hr/ml, 893.7 ng.hr/ml, 0.177 $hr^{-1}$, 3.70 hr., 0.558 $hr^{-1}$ , 1.59 hr.
أما للدراسة ألثانية مع ألطعام فالنتائج للمنتج ألجنيس هى:
157.8 ng/ml, 826.5 ng.hr/ml, 853.8 ng.hr/ml, 0.198 $hr^{-1}$, 5.4 hr., 0.458 $hr^{-1}$ , 2.06 hr.;
وللمنتج ألمرجعى هى:
162.1 ng/ml, 869.7 ng.hr/ml, 894.8 ng.hr/ml, 0.196 $hr^{-1}$, 4.1 hr., 0.525 $hr^{-1}$, 1.80 hr.
بناء على ألدستور ألأمريكى وألأوربى فان ألنتائج ألمذكوره أعلاه تدل على وجود تكافؤ حيوى بين ألمنتجين عند تناول ألدواء مع ألطعام وبدون طعام. لذلك فمن الممكن ألأستنتاج بان ألمنتج طويل ألمفعول (ER) ألمطور حديثا ألذى يحتوى على ٤٠٠ ملغ من البنتوكسيفلين مكافؤ حيويا مع ترنتال ٤٠٠ ملغ طويل ألمفعول (ER) المنتج من شركة سنوفى أفينتس.

**الكلمات ألآفتتاحية:- بنتوكسيفلين , حركية الدواء , ألتكافؤ ألحيوى .**

## Introduction

Pentoxifylline (Pentoxiphylline) is a tri-substituted xanthine derivative designated chemically as 1-(5-oxohexyl)-3, 7-dimethylxanthine that, unlike theophylline, is a hemorrheologic agent, i.e. an agent that affects blood viscosity. Pentoxifylline is indicated for the treatment of patients with intermittent claudication on the basis of chronic occlusive arterial disease of the limbs. After administration of the 400 mg extended-release (ER) pentoxifylline tablet, plasma levels of the parent compound and its metabolites reach their maximum within 2 to 4 hours and remain constant over an extended period of time. Coadministration of ER pentoxifylline tablets with meals resulted in an increase in mean $C_{max}$ and AUC by about 28% and 13% for pentoxifylline, respectively. The usual dosage of ER pentoxifylline tablet form is one tablet (400 mg) three times a day with meals [(1)] .

The aim of the present study is to evaluate the bioequivalence of a newly developed extended - released (ER) tablet containing 400 mg pentoxifylline relative to the reference brand Trental® ER tablet containing 400 mg pentoxifylline manufactured by Sanofi-Aventis. As per FDA and EMEA Guidance for bioequivalence evaluation of modified-release products [(2-4)], two separate studies are required; a single dose, nonreplicate, fasting study and a single dose, food-effect, nonreplicate study. Therefore, the pharmacokinetic parameters $C_{max,}$ $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$ , $T_{max}$, $\lambda_Z$ & $T_{0.5}$ were calculated in the current investigation for both products after administration to 32 healthy male adult subjects under fasting and fed conditions.

## Materials and Methods

### *Drug products*

The test product was a newly developed extended-release (ER) tablet containing 400 mg pentoxifylline. The reference product was the brand Trental® ER tablet containing 400 mg pentoxifylline manufactured by Sanofi-Aventis.

### *Study design*

As recommended by FDA and EMEA guidance concerning the bioequivalence of modified released drug products [(2-4)], two separate bioequivalence studies are required to be conducted to prove bioequivalence of a test product to the reference/brand/innovator product. These studies include a single dose, nonreplicate bioequivalence study under fasting condition, and a single dose, nonreplicate bioequivalence study under fed condition. Accordingly, two bioequivalence studies were conducted in the present investigation: a fasting, single-dose, two-treatment, two-period, two-sequence, randomized crossover study, and a fed single-dose, two-treatment, two-period, two-sequence, randomized crossover study. Thirty two subjects participated in each study. In each study, equal number of subjects (16 subjects) were randomly assigned to each dosing sequence of the treatments (test and reference formulations). The treatments were separated by one week washout interval between period I and period II dosing.

### *Inclusion criteria of volunteers*

The volunteers were selected according to the following inclusion criteria: male, age between 18-45 years, normal body mass index, non-smokers or light smokers (less than 10 cigarettes a day), normal physical examinations, no clinically medical disorders or impairments (hepatic, renal, cardiac, GIT and psychiatric), no history of contraindication and/or allergy to the drug or any related compounds, no consumption of drugs for two weeks prior the study, OTC drugs are allowed as per clinical investigator decision. The volunteers should not have been participated in clinical study (bioavailability, bioequivalence, pharmacokinetics, etc.) studies 3 months prior

to the present study, no blood donation or hospitalization 3 months prior to the present study, no drugs or alcohol abuse, normal laboratory tests including biochemistry, hematology, HIV (-), hepatitis B and C (-), liver and kidneys function tests and urine analysis.

***Informed consent of volunteers***

The study was conducted according to ICH guidelines for good clinical practice (GCP) and declaration of Helsinki [5, 6]. According to Helsinki Declaration, the informed consent form includes details of the study, benefits and possible risks associated with participation, information regarding the right to withdraw at any time from participation. The informed consent form was provided to each prospective volunteer prior to the start of the study. Also, a study-orientation session was held with the volunteers to explain and inform the details of the informed consent form. All the volunteers gave written and signed consent before the study.

***Conditions of the clinical study for fasting and fed studies***

***In case of fasting study***; the volunteers were confined at the clinical site 14 hours before dose administration and until 24 hours after dose administration (end of each study period). A standard dinner was served to the volunteers 12 hours before dosing. The drug was administered with 240 ml of water after an overnight fasting of 12 hours. Mouth checks and hands checks were performed by the investigators to ensure that the medication is taken by the volunteers as directed. Four hours after dosing, a standard lunch was served to the volunteers. Food and the time of feeding were identical in all periods of the study. No water was permitted 2 hours before and after dosing. Water was allowed as desired 2 hours after dosing. Xanthine containing products were not allowed 12 hours before dosing and until 24 hours after dosing (end of each study period). The volunteers were not allowed to sleep or lie during the first four hours of drug administration. Grapefruit juice or beverages containing grapefruit were not allowed within the past week prior the study and until the completion of the whole study (both periods of the study).

***In case of fed study***; the same procedure was applied as mentioned above except the volunteers were served standard fatty breakfast before drug intake as per FDA and EMEA guidance [2, 3] .

***Blood sampling from volunteers***

Seven ml of blood samples were withdrawn from each volunteer via an Indwelling cannula placed in the forearm anticubital vein. The cannula was kept patent by flushing with 1 ml of heparinized saline (2 IU per ml) after each sample collection. About 0.2 ml of blood was discarded from the cannula before each sampling withdrawal. The blood was sampled from each volunteer at zero time (30 min. before dosing), and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0 and 24.0 hours post dosing (a total of 22 blood samples were collected from each volunteer). The blood samples were transferred to heparinized tubes and then immediately centrifuged for 10 minutes at 4000 rpm. Blood and plasma samples were labeled according to in-house coding system. The labeling system is confidential and the analysts have no key to the labeling system. The plasma was separated by polypropylene disposable tips and transferred to eppendorf tubes and then stored in deep freezer at −20°C until the time of analysis for determination of pentoxifylline concentrations.

***Medical observation during and post dosing***

The clinical staff were available during each study period to conduct the study as per study protocol, and to treat and report any adverse effect if any. Vital signs (blood pressure and pulse) of each subject were measured one hour before dosing and then at 1, 2, 3, 6, 9, 12, and 24 hours post dosing (the end of each study period). The volunteers were free to leave the study at any time for any reason. Withdrawal to protect the health of the volunteers, if any, was also considered.

***Data analysis for pharmacokinetic (PK) calculations:***

Kinetica software was applied for all PK calculations, and data plotting. The PK parameters; $C_{max,}$ $AUC_{0-t}$ , $AUC_{t-\infty}$ , $AUC_{0-\infty}$ , $C_{max}/AUC_{0-\infty}$ , $T_{max}$ , $\lambda_Z$ and $T_{0.5}$ were calculated for each subject and for each period applying non-compartmental analysis [7, 8]. Ln-transformation of the primary PK parameters used for bioequivalence testing $C_{max,}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were also computed as recommended by FDA and EMEA guidance [2-4] . The terminal elimination rate constant ($\lambda_Z$) was estimated for each subject and for each period via linear regression of the last points (at least three points) of the terminal phase of the log-concentration versus time curve [2-4]. The values of $C_{max}$ and $T_{max}$ were obtained directly from concentration versus time curves of each individual. Mean drug concentrations in plasma vs. time data for both test and reference products were plotted in rectilinear graph type.

***Statistical analysis of the pharmacokinetic (PK) parameters:***

The same software Kinetica that was used for PK calculations was also applied for all statistical analysis. The parameters $C_{max,}$ $AUC_{0-t}$ , $AUC_{t-\infty}$ , $AUC_{0-\infty}$ , $C_{max}/AUC_{0-\infty}$ , $T_{max}$ , $\lambda_Z$ and $T_{0.5}$ were statically analyzed to evaluate the differences between the test and the reference products applying descriptive statistics, ANOVA test, and 90% confidence interval (CI). Descriptive statistics included arithmetic means, geometric means, ratio of geometric means, and standard deviation. ANOVA test was applied to calculate the effect of the factors; period, subjects nested in sequence, treatment (formulation) and sequence; on the above mentioned PK parameters. ANOVA test was also applied to the Ln-transformed values of $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ .

The two products were declared bioequivalent if the CI of ratio of the test product to reference product (T/R) of the Ln-transformed parameters $C_{max}$ , $AUC_{0-t}$ and $AUC_{0-\infty}$ lie between 80 to 125% [(9)] . Differences are declared statistically not significant at 5% significance level ($\propto = 0.05$) when $P \geq 0.05$.

***Determination of pentoxifylline in plasma***

A specific High Performance Liquid Chromatographic (HPLC) assay using UV detection and chloramphenicol as internal standard was developed in-house for determination of penotxifylline in plasma. The lower limit of quantitation (LLOQ) of penotxifylline in plasma was 5 ng/ml and the upper limit of quantitation (ULOQ) was 500 ng/ml. The analytical method was validated according to FDA bioanalytical method validation guidelines [(10)]. All plasma samples of each volunteer obtained from both periods (test and reference products) were analyzed together with quality control (QC) samples (low, medium & high) in one analytical run (batch). A standard curve including blank matrix was generated for each analytical run and was used to determine penotxifylline concentrations in the unknown authentic samples. No determination was done by extrapolation below the LLOQ or above the ULOQ of the standard calibration curve. The plasma samples were analyzed after the completion of the clinical part of the study.

***Products assay***

Assay determination of penotxiphylline in the test product and the reference product were carried out to insure that the difference in the content of the drug in the test product and the reference product is not more than 5% as recommended by FDA and EMEA guidance [(2-4)].

***Dissolution testing***

The dissolution testing of the test product and the reference product was carried out to study the similarity in the dissolution profiles between both formulas by calculating the similarity factor F2 as per FDA and EMEA guidance [(2, 4, 11)] .

## Results and Discussions

***Clinical data***

Tables 1and 2 presents the demographic data of the volunteers participated in the fasting study (32 subjects) and the fed study (32 subjects), respectively.

**Table (1): Demographic data of 32 volunteers participated in the fasting study.**

| Volunteers | Mean | ±SD | %CV |
|---|---|---|---|
| Age (years) | 34.9 | 7.1 | 20.3 |
| Weight (kg) | 65.1 | 8.4 | 12.9 |
| Height (cm) | 173.8 | 8.9 | 05.1 |

**Table (2): Demographic data of 32 volunteers participated in the fed study.**

| Volunteers | Mean | ±SD | %CV |
|---|---|---|---|
| Age (years) | 36.1 | 8.9 | 24.6 |
| Weight (kg) | 64.3 | 9.8 | 15.2 |
| Height (cm) | 172.2 | 7.9 | 04.6 |

It is obvious from Tables 1 and 2 that the descriptive statistics of the demographic data of the volunteers selected for fasting study was almost similar to the fed study in order to exclude the potential difference in the pharmacokinetics of drug due to age, weight and/or height.

All the volunteers started each study (fasting or fed) completed both periods of the study, i.e., no drop out or withdrawal were reported for both studies. Both test and reference products were well tolerated by all volunteers. No incidence of side effects or adverse reactions were observed during the entire study. Beside, all the volunteers left the study without any change in their base line condition (vital signs).

***Products assay***

Assay of penotxifylline in the test product and the reference product showed that the drug content (Penotxifylline) of the test product differs by less than 5% from that of the reference product, thus conform with the FDA and EMEA requirement [(2-4)].

***Dissolution testing***

Evaluation of the dissolution data of both products based on FDA and EMEA

guidance [2-4] indicate that the dissolution data of both products are similar and insure sameness or equivalence of both products since the similarity factor (F2) value was 95.6%.

***Plasma concentrations of pentoxifylline vs. time data for fasting and fed studies***

Thirty minutes before dosing (at zero time) of each product intake and for both fasting and fed studies, penotxifylline was not detected in plasma in all volunteers and in both periods of the study, which insure the absence of carryover effects. The drug was detected in plasma samples of all the 32 volunteers after 0.5 hour of both products intake and after both fasting and fed studies. This finding indicates rapid absorption of the drug from the test and the reference ER form of pentoxifylline tablets. The levels of pentoxifylline were not detected in plasma after 20 hrs (below the LLOQ 5 ng/ml) post dosing of the test and reference products and in both fasting and fed studies.

Figures 1 and 2 show mean plasma concentrations of pentoxifylline versus time after a single dose administration of a test formulation and the reference formulation under fasting and fed conditions, respectively. Both figures 1 and 2 show a very good agreement between the mean plasma concentration-time profiles of both products.

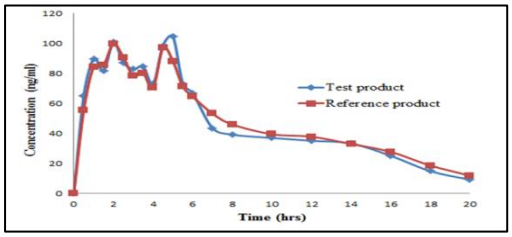
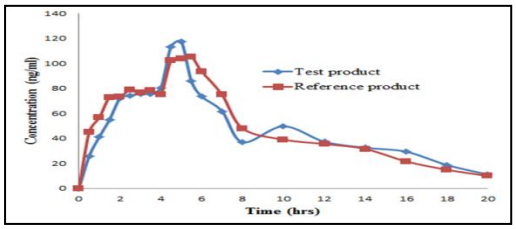



**Figure (1): Mean plasma concentrations of pentoxifylline versus time after a single dose administration of a test formulation (extended-release tablet containing 400 mg pentoxifylline) and the reference formulation (Trental® extended-release tablet containing 400 mg pentoxifylline) to 32 healthy male adult subjects under fasting condition.**




**Figure(2): Mean plasma concentrations of pentoxifylline versus time after a single dose administration of a test formulation (extended-release tablet containing 400 mg pentoxifylline) and the reference formulation (Trental® extended-release tablet containing 400 mg pentoxifylline) to 32 healthy male adult subjects under fed condition**.

Using ANOVA test, the plasma concentrations of penotxifylline at each time point for the test product were statistically compared against the corresponding plasma concentrations, at the same time points for the reference product. It appeared from ANOVA test that there is no significant difference in the concentration-time profiles of both products at each time point and in both fasting and fed state. Thus, there is good similarity in the concentration-time profiles between the test and the reference formulas, and under both fasting and fed conditions.

***Pharmacokinetic parameters of pentoxi - fhylline for fasting and fed studies***

Tables 3 and 4 show the pharmacokinetic parameters of pentoxifylline for both test and reference products under fasting and fed conditions, respectively.

***For fasting study***; the mean values of the pharmacokinetic parameters $C_{max}$ , $AUC_{0-t}$ , $AUC_{0-\infty}$ , $C_{max}/AUC_{0-\infty}$ , $T_{max}$, $\lambda_Z$ & $T_{0.5}$ for the test formula were; 144.4 ng/ml, 845.4 ng.hr/ml, 868.4 ng.hr/ml, 0.186 $hr^{-1}$, 3.29 hr, 0.561 $hr^{-1}$ and 1.65 hr, respectively. The mean values of these parameter for the reference formula were; 150.0 ng/ml, 871.1 ng.hr/ml, 893.7 ng.hr/ml, 0.177 $hr^{-1}$, 3.70 hr, 0.558 $hr^{-1}$ and 1.59 hr, respectively (Table 3). It is obvious that there is good similarity in the pharmacokinetics of the reference and the test product. Concerning the parameter $C_{max}$, different values were reported in literature. A study reported a mean $C_{max}$ of 541.0 ng/ml [12]. Other study [13] reported different mean $C_{max}$ values at two different drug administration times of the day, in which the mean $C_{max}$ was 326.4 ng/ml following morning (10:00 hr) administration and 266.4 ng/ml following night (22:00 hr) administration [13]. More recent studies [14-17] reported mean $C_{max}$ values of 166.9 ng/ml, 218.4 ng/ml, 67.9 ng/ml and 132.6 ng/ml, respectively. Thus, the reported studies indicate that the mean $C_{max}$ values are variable among populations and time of drug administration [12-17]. This investigation (Table 3) preset mean $C_{max}$ closer to references [14-17]. Regarding the parameter AUC, a previous study [12] reported a mean value of 1422.0 ng.hr/ml. Other study [13] reported different mean AUC values at two different drug administration times of the day, in which the mean AUC was 2424.0 ng.hr/ml following morning (10:00 hr) administration and 2124 ng.hr/ml following night (22:00 hr) administration [13]. More recent investigations [14-17], reported mean values of 1078.2 ng.hr/ml, 1528.9 ng.hr/ml, 1270.0 ng.hr/ml and 1104.1 ng.hr/ml, respectively. The present investigation elucidated mean AUC almost

similar to that reported in the studies [14-17]. Thus, similar to the parameter $C_{max}$ , the parameter AUC exhibit variable results between populations and time of drug administration. For the parameter $T_{max}$ , the reported mean values ranged from 1 to 4 hrs [1, 12, 14-17]. Almost similar result was obtained in the present study (Table 3). Regarding the parameter $T_{0.5}$, the mean values were found to be 1.32, 2.87 and 1.84 hrs, respectively [12, 14, 16] which is in a good agreement with the mean value found in the present study (Table 3). The ratio $C_{max}$ /$AUC_{0-\infty}$ which is considered as an appropriate measure for evaluating drug absorption in bioequivalence testing [18-20] supports the similarity in the absorption rate of both products (Tables 3). This in turn suggests similar absorption behavior of the two formulations in the GIT.

***For the fed study***; the mean values of the parameters $C_{max}$ , $AUC_{0-t}$ , $AUC_{0-\infty}$ , $C_{max}/AUC_{0-\infty}$ , $T_{max}$, $\lambda_Z$ and $T_{0.5}$ for the test formula were; 157.8 ng/ml, 826.5 ng.hr/ml, 853.8 ng.hr/ml, 0.198 $hr^{-1}$, 5.4 hr, 0.458 $hr^{-1}$ and 2.06 hr, respectively. The mean values of these parameter for the reference formula were; 162.1 ng/ml, 869.7 ng.hr/ml, 894.8 ng.hr/ml, 0.196 $hr^{-1}$, 4.1 hr, 0.525 $hr^{-1}$ and 1.80 hr, respectively (Table 4). The above mentioned data indicate that the pharmacokinetic characteristics of the drug are similar for both products. Comparison of these values (Table 4) with those observed after fasting condition (Table 3) show about 10% increase in $C_{max}$ and 70% increase in $T_{max}$ after food intake. Whereas, food intake demonstrated no significant changes in other studied pharmacokinetic parameters (Tables 3 and 4). A literature [1] reported that coadministration of ER pentoxifylline tablets with meals resulted in an increase in mean $C_{max}$ and AUC by about 28% and 13%, respectively.

The LLOQ of 5 ng/ml and ULOQ of 500 ng/ml applied in the current investigation are quiet enough for pharmacokinetics, bioavailability and bioequivalence studies of pentoxifylline ER tablets. Beside, 24 hrs blood sampling and one week washout period between dosing used in this study are adequate to ensure almost complete removal of the drug from the body and consequently prevent carryover effects.

**Table (3): Pharmacokinetic parameters of pentoxifylline after a single dose administration of a test formulation (extended- release tablet containing 400 mg pentoxifylline) and the reference formulation (Trental® extended- release tablet containing 400 mg pentoxifylline) to 32 healthy male adult subjects under fasting condition. Mean ± SD**

| Pharmacokinetic Parameters | Test Formula | | Reference Formula | |
|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD |
| **$C_{max}$ (ng/ml)** | 144.4 | 79.2 | 150.0 | 94.0 |
| **$AUC_{0-t}$ (ng.hr/ml)** | 845.4 | 481.8 | 871.1 | 441.4 |
| **$AUC_{0-\infty}$ (ng.hr/ml)** | 868.4 | 486.9 | 893.7 | 445.1 |
| **$C_{max}$ /$AUC_{0-\infty}$ ($hr^{-1}$)** | 0.186 | 0.058 | 0.177 | 0.054 |
| **$T_{max}$ (hr)** | 03.29 | 1.895 | 03.70 | 2.553 |
| **$\lambda_Z$ ($hr^{-1}$)** | 0.561 | 0.304 | 0.558 | 0.214 |
| **$T_{0.5}$ (hr)** | 01.65 | 0.641 | 01.59 | 0.654 |

$C_{max}$ = Maximum concentration of drug in plasma.

$T_{max}$ = Time to attain $C_{max}$.

$AUC_{0-t}$= Area under plasma concentration-time curve from time zero to $t_{last}$, calculated by trapezoidal rule.

$AUC_{t-\infty}$ = Extrapolated area under plasma concentration-time curve from $t_{last}$ to infinity, calculated as $C_{last}/\lambda_Z$.

$AUC_{0-\infty}$ = Total area under plasma concentration-time curve from time zero to infinity, calculated from the sum of $AUC_{0-t}$ + $AUC_{t-\infty}$.

$\lambda_Z$ = First order terminal elimination rate constant

$T_{0.5}$ = First order terminal elimination half-life, equal to $0.693/\lambda_Z$.

$C_{last}$ = Last measurable concentration which meet or exceed the lower limit of quantitation.

$t_{last}$ = Time at which $C_{last}$ occur.

**Table (4): Pharmacokinetic parameters of pentoxifylline after a single dose administration of a test formulation (extended- release tablet containing 400 mg pentoxifylline) and the reference formulation (Trental® extended-release tablet containing 400 mg pentoxifylline) to 32 healthy male adult subjects under fed condition. Mean ± SD.**

| Pharmacokinetic Parameters | Test Formula | | Reference Formula | |
|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD |
| $C_{max}$ (ng/ml) | 157.8 | 105.0 | 162.1 | 94.0 |
| $AUC_{0-t}$ (ng.hr/ml) | 826.5 | 537.0 | 869.7 | 468.1 |
| $AUC_{0-\infty}$ (ng.hr/ml) | 853.8 | 537.4 | 894.8 | 472.7 |
| $C_{max}/AUC_{0-\infty}$ ($hr^{-1}$) | 0.198 | 0.070 | 0.196 | 0.078 |
| $T_{max}$ (hr) | 05.4 | 03.05 | 04.1 | 01.89 |
| $\lambda_Z$ ($hr^{-1}$) | 0.458 | 0.223 | 0.525 | 0.192 |
| $T_{0.5}$ (hr) | 02.06 | 0.945 | 01.80 | 1.187 |

***Statistical analysis of the pharmacokinetic parameters for fasting and fed studies***

For the fasting study; ANOVA test for the pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, $T_{max}$, $\lambda_Z$ & $T_{0.5}$ of the test product versus the reference product exhibited no significant differences ($P \geq 0.05$). Beside, no significant differences ($P \geq 0.05$) was found in the above mentioned parameters for the fed study between the test and the reference products. The geometric mean ratio and the 90% confidence interval demonstrated bioequivalence of both products under fasting condition (Table 5) and fed condition (Table 6). Therefore, according to FDA and EMEA guidance in bioavailability and bioequivalence [2-4], it can be concluded that the newly developed pentoxifylline ER 400 mg tablets are bioequivalent to the reference brand product Trental ER 400 mg tablets produced by Sanofi-Aventis under both fasting and fed states.

**Table (5): Geometric mean ratio and 90% Confidence Interval for the pharmacokinetic Parameters of the test versus the reference products in fasting state**.

| Pharmacokinetic Parameters | T/R Geometric Mean Ratio | 90% Confidence Interval* | |
|---|---|---|---|
| | | Lower limit | Upper limit |
| $C_{max}$ | 0.94 | 87.9 | 111.2 |
| $AUC_{0-t}$ | 0.95 | 85.7 | 106.5 |
| $AUC_{0-\infty}$ | 0.95 | 86.4 | 107.8 |

*Acceptance criteria = lower limit ≥ 80 and upper limit ≤ 125.0.

**Table (6): Geometric mean ratio and 90% Confidence Interval for the pharmacokinetic Parameters of the test versus the reference products in fed state.**

| Pharmacokinetic Parameters | T/R Geometric Mean Ratio | 90% Confidence Interval* | |
|---|---|---|---|
| | | Lower limit | Upper limit |
| $C_{max}$ | 0.95 | 81.7 | 112.8 |
| $AUC_{0-t}$ | 0.92 | 84.1 | 104.1 |
| $AUC_{0-\infty}$ | 0.92 | 85.6 | 104.2 |

*Acceptance criteria = lower limit ≥ 80 and upper limit ≤ 125.0.

## Conclusion

The present investigation presents the pharmacokinetics of pentoxifylline ER 400 mg tablets under fasting and fed conditions. Beside, food intake demonstrated no significant changes in the pharmacokinetic characteristics of the drug. Plasma concentrations and consequently the pharmacokinetic of the drug can be determined in human applying HPLC/UV method. The newly developed pentoxifylline 400 mg ER tablets are bioequivalent to the innovator brand product Trantal 400 mg ER tablets in term of rate and extent of bioavailability. Therefore, the newly developed product is interchangeable with Trental ER 400 mg tablet and can be prescribable in medical practice.

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