

# Comparative Pharmacokinetics, Safety and Tolerability Evaluation of Acyclovir IR 800 Mg Tablet in Healthy Indian Adult Volunteers Under Fasting and Non-fasting Conditions

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## Abstract

**Objectives:** This study was designed to determine the comparative pharmacokinetics, safety and tolerability of test acyclovir 800mg IR formulation with reference product (Zovirax® 800mg IR Tablet) after single dose administration under fasting and non-fasting conditions in 36 male and female adult subjects.

**Methods:** Open label, balanced, randomized, two-sequence, single-dose, two-way crossover study design in healthy Indian adult volunteers with a washout period of at least 7 days was used. Each subject received an acyclovir test or reference product respectively. Blood samples were collected before dosing and at various time points up to 24 hours after dosing. Plasma samples were analyzed by validated liquid chromatography with tandem mass spectrometry method. The pharmacokinetic parameters  $C_{max}$ ,  $T_{max}$ , MRT,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were analyzed using non-compartment model. Drug safety and tolerability were assessed.

**Results:** Total 36 and 34 subjects in fasting in non-fasting study had completed both treatment periods. Two subjects were dropout due to family reason. No statistical significance difference of pharmacokinetic parameters  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  between test and reference product. Food dose not affects the rate and extent of acyclovir absorption in systemic circulation was observed in both fasting and non-fasting study. The significant sequence (carry-over) effects of  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were acceptable due to non detectable drug in pre-dose samples in analyzed subjects. Total 07 subjects reported 26 adverse events during the fasting study, and 06 subjects reported adverse events during the non-fasting study. Acyclovir was found safe and well tolerated by all subjects who had completed the study in good health conditions.

**Conclusion:** The single dose pharmacokinetics, safety and tolerability study found that the test formulation acyclovir 800mg immediate release tablets were comparable to the reference product at rate and extent of absorption under fasting and non-fasting conditions in healthy adult subjects according to the USFDA regulatory guidance.

**Keywords:** Acyclovir; Pharmacokinetics; Bioequivalence; Safety and Tolerability

## Introduction

Acyclovir is a synthetic purine nucleoside analogue with *in vitro* and *in vivo* inhibitory activity against herpes simplex virus types 1 (HSV-1), 2 (HSV-2) [1-7], and varicella-zoster virus (VZV) [8-13]. The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. The greater antiviral activity of acyclovir against HSV compared with VZV is due to its more efficient phosphorylation by the viral TK. Acyclovir has been reported as a potent and selective inhibitor of the replication of HSV and, to a lesser extent, of VZV. Acyclovir became the drug of choice for the treatment of HSV and VZV infections, particularly primary and recurrent genital herpes and mucocutaneous HSV [2-7,12] and VZV infections in immunosuppressed patients [5-7,8-14].

Acyclovir is commonly used as the free acid form in solid dosage forms, whereas the sodium salt is used in parental dosage forms [15,16]. Acyclovir is "slightly soluble in water" at room temperature (22-25°C) in different Pharmacopoeias and solubility values range from 1.2 to 1.6 mg/mL [17-22]. The solubility was vary slightly with pH and lowest solubility of 2.3 mg/mL at pH 5.8 at 37°C. The partition coefficient (log *P*) in n-octanol at 25°C was -1.59 [23]. Acyclovir is an ampholyte with both weak acid and basic groups with reported pK<sub>a</sub> values of 2.16 and 9.04 at 37°C [24].

The drugs with permeability in the range 70-100% absorbed usually

have a  $P_{app}$  value greater than  $10 \times 10^{-6}$  cm/s, but acyclovir permeability coefficient ranging from  $0.12 \times 10^{-6}$  to  $2.0 \times 10^{-6}$  cm/s suggest that permeability of the acyclovir is low [19,25]. The log *P* value greater than that of metoprolol (1.72) indicates high permeability, but acyclovir log *P* values reported (-1.59) indicating as expected to low permeability. [23] The various excipients permeability study with Caco-2 showed no effect on the permeability in acyclovir immediate release (IR) solid oral drug products [26].

Absorption of acyclovir from the gastrointestinal tract is variable and incomplete; 10-30% of an oral dose may be absorbed [15-18]. This poor systemic bioavailability is considered to be a result of the characteristics of the drug itself and not its delivery vehicle [4]. Because of its high hydrophilic nature, absorption of acyclovir occurs mainly by passive

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diffusion mechanism and it is slow, variable and incomplete manner [4,16]. Therefore, acyclovir is replaced with its prodrug valacyclovir, the L-valyl ester of acyclovir has been used orally to increase the systemic bioavailability [15,16]. Peak plasma concentrations usually are attained within 1.5–2.5 hours after oral administration [15,16]. Acyclovir shows a two compartment pharmacokinetic behavior, regardless of the dosage, duration of the treatment or frequency of administration. [27] After multiple dose administration, steady-state concentrations are reached in 1-2 days [28,29]. The increasing acyclovir doses results in decreasing bioavailability due to saturable carrier system or a limited area for absorption in the gastro-intestinal (GI) tract [21]. The increase in doses (dose range used clinically of 100-800mg) proportionally increase in AUC [22,30]. Food does not appear to affect GI absorption [4,16,28,29]. Acyclovir is widely distributed into most body tissues including the brain, kidney, lung, liver, heart, tissue, muscle, spleen, placenta, uterus, vaginal mucosa and secretions, semen, saliva, aqueous humour and cerebrospinal fluid [15]. Acyclovir demonstrates minimal protein binding (9-33%) at therapeutic plasma concentrations [15].

Acyclovir is metabolized partially to 9-carboxymethoxymethylguanine also converted intracellularly in cells infected with herpesviruses to acyclovir triphosphate, the pharmacologically active form of the drug [30-32]. Acyclovir is excreted principally in urine as unchanged drug with initial serum half-life averages 0.34 hour and terminal half-life averages 2.1–3.5 hours. The main excretory organ for acyclovir is the kidney [28]. The plasma half-life of oral acyclovir on average is 3 hours in adults with normal renal function [28,33]. Approximately 80% of an oral dose is never absorbed and is excreted through the faeces [28-34].

Acyclovir is well tolerated whether administered by ocular, topical, oral or intravenous routes [32,35-38]. The incidences of most adverse events, such as topical preparations have been mainly limited to mild local effects [39,40]. The incidence of most adverse events such as gastrointestinal symptoms, rash and headache, occurring during oral acyclovir is similar to that seen in patients receiving placebo [41]. There have been occasional reports of acute, usually reversible, renal failure and neurotoxicity associated with the oral formulations, but occurrence more often with intravenous administration, usually in patients with high peak plasma acyclovir concentrations [37,42-44].

The scope of this article is restricted to drug products containing acyclovir as the free acid. Literature survey revealed that acyclovir is well tolerated, but mild gastrointestinal effects may occur with the oral formulations in a few patients, and acute reversible renal failure and neurotoxicity has been reported with high peak plasma acyclovir concentrations, usually in patients receiving intravenous administration [32,37-44]. Because of incomplete and variable absorption of acyclovir, the present study involves development of a newly immediate release oral highest dose (800mg) pharmaceutical formulation used clinically significant in various HSV and VZV infected patients. This study was designed to assess comparative pharmacokinetics, food effects, safety and tolerability of newly developed oral test immediate release formulation with that of a reference product (Zovirax<sup>®</sup>, GlaxoSmithKline, Uxbridge, UK) in healthy, male, Indian volunteers under fasting and non-fasting conditions according to ICH guidelines [45,46].

## Participants and Methods

### Study subjects

The study protocol was approved by the Institutional Ethics Committee (IEC) and study was conducted in accordance with Good

Clinical Practice (GCP) and the guidelines of the Helsinki Declaration 2008. Thirty six healthy volunteers (18 male and 18 female) were scheduled to participate in each fasting and non-fasting study. Subjects were undergoing a screening procedure at least 21 days before the first day of dosing. All the subjects were provided and obtained written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study periods.

Subjects were eligible to participate if they were 18 to 45 years of age, Body Mass Index (BMI) 18.0 to 25.0 Kg/m<sup>2</sup>, willingness to sign statements of written informed consent and adhere to protocol entire study period, non-smoker or mild to moderate smokers (3-5 cigarettes/biddies/pipes daily), and tobacco consumption ( $\leq$ 2-4 packets daily) and willing to discontinue smoking and tobacco consumptions 48 hours before initiation of study and during the study period. Subjects were excluded based on history of alcoholism (>600mL weekly) or drug addiction, high consumption of stimulating drinks, consumption of medications that could alter the acyclovir metabolism during previous month and Over-the-Counter (OTC) drugs during two previous week, history of illness or major surgery during last two months, blood donation since last two months, medication allergy, illness or disorders that affect the absorption, distribution, metabolism and/or excretion of drugs, histories of adverse reactions to acyclovir or related drugs, presence of disease marker of HIV 1 and 2, Hepatitis B & C viruses, positive test of urine drug of abuse (Amphetamines, Morphine, Benzodiazepines, Marizuana, Cocain and Barbiturate), positive urine pregnancy test (female only), abnormal hematological and biochemical laboratory parameters test, abnormal ECG and X-ray findings and vital signs or clinical examination. The subjects were agreed to refrain from the use of prescription or nonprescription drugs (including vitamins), xanthine containing food or beverages (tea, coffee, chocolates, soft drinks like cola etc.) and alcoholic product consumption within 48 hours and grape fruit or juice or metabolite within 72 hours prior to the dosing in each period and during the entire study period.

The subjects were withdraw from study due to suffering significant inter-current illness or undergoes surgery during the course of study, violation of the protocol, concomitant medication which interferes acyclovir pharmacokinetic property, subject willingness his/her own to withdrawn consent, experience adverse events for best interest of the subject, emesis occurs at or before 2 times median  $T_{max}$  and found positive in urine pregnancy test (female only).

### Acyclovir tablet formulation

The ingredients used for test acyclovir 800mg immediate release tablet formulations were: acyclovir 800mg, micro-crystalline cellulose (Avicel<sup>®</sup> PH101), sodium starch glycolate, FD&C Blue No. 2, purified talc and magnesium stearate. The reference product was Zovirax<sup>®</sup> 800mg immediate release tablet. The ingredients used in the reference product (according to the information provided by the company) were: micro-crystalline cellulose, sodium starch glycolate, FD&C Blue No. 2, providone and magnesium stearate.

### In vitro dissolution study

**Acyclovir test formulation:** The *in vitro* dissolution and disintegration behaviors of test acyclovir 800mg immediate release formulation was studied in various dissolution media as mentioned below and *in vivo* in comparison against reference product (Zovirax<sup>®</sup> IR 800mg tablets) [50-51]. This reference product was subsequently used as clinical trial materials for the pharmacokinetics study in human volunteers. The physical properties, weight variation, thickness, diameter and hardness test of tablet formulation was conducted in

accordance with the current compendial criteria [50]. The friability and content uniformity of formulated tablet was determined based on the USP 27 [51]. The pH-solubility of acyclovir test formulation was investigated for its solubility in various medium e.g., 0.1 N HCl (pH 2.2), pH 1.2 in SGF<sub>sp</sub> (simulated gastric fluid without enzymes), phosphate buffer pH 4.5, pH 5.0 in FeSSIF (fed state simulated Intestinal fluid), pH 6.8 in SIF<sub>sp</sub> (simulated Intestinal fluid without pancreatine) and deionized water (pH approx. 5.5) for fasted and non-fasted study evaluation according to the BCS criteria within different physiological conditions (pH range) for the stimulation of gastrointestinal tract [47-49]. The disintegration testing of each 12 test formulation and reference product were measured in different media (mentioned above) by using the disintegration test apparatus USP for 30 minutes. The tablets were considered completely disintegrated when all the particles have passed through the mesh. The dissolution testing of each 12 test formulation and reference product were performed using USP Apparatus II (paddle method) with the revolution of 50 rpm in different media (mentioned above) in order to evaluate the dissolution behavior of pure drug substance without the effect of inactive ingredients at 37±5°C. Aliquots of the dissolution medium were removed at 15,30,45,60 and 90 minutes, respectively. The percent of drug dissolved at each sampling time point was determined by UV spectrophotometric and HPLC analysis (for SIF<sub>sp</sub> medium only) at the wavelength of 260 nm [47-49]. The significance (p<0.05) dissolution profile of each 12 test formulation and reference product in various dissolution media were compared in accordance with the “model independent approach” using a difference factor (f1<15) and a similarity factor (f2>50) which is one of the dissolution profile comparison methods recommended by the USFDA [50].

**Commercially available acyclovir 800mg IR tablet:** The dissolution behaviors of all commercially available acyclovir 800mg IR tablet were evaluated for their release patterns and whether they were fall within the “rapidly dissolving” range of the BCS criteria [47-49]. Ten brands of commercially available immediate release acyclovir 800mg tablets in different zone of India were selected and evaluated for their release characteristics e.g. Acvirax (Novus Life Sciences Pvt Ltd), Ocuvir (FDC Limited), Zovir (East West Pharma), Virclo (Shinto Biotec Ltd), Clyvir (Welbe Life Sciences), Alovir (Adley Formulation), Herperax (Gratia, Micro Labs Ltd), Rovir (Royal Labs), Zovirax (Glaxo SmithKline Pharmaceuticals Ltd.) and Lovir (Eli Lilly and Company, India, Pvt Ltd), respectively. Ten commercial brands each 12 tablets were tested by using USP Apparatus II with dissolution media mentioned above representing acidic and basic environment in accordance with human GI physiology (to stimulate intestinal conditions in the fasted and fed state), respectively. The media volume applied was 900 ml with the paddle agitation speed of 50 rpm at 37±5°C. Aliquots of the dissolution medium were removed at 15,30,45,60 and 90 minutes, respectively. Cumulative acyclovir concentration expressed as a percentage of the labeled claim from each sampling time point was analyzed for drug content as dissolution profiles [47-49].

### Subject sample size determination

Subject sample size was based on estimates obtained from reported literature/previous pilot study (both fasting and non-fasting conditions). Assuming a true Test/Reference ratio between 95 and 105% and an intra-subject variability (CV%) of ≈22.89%, with 5% significance level and power >90%, at a minimum of 34 subjects was required to conclude bioequivalence limit between 80 and 125%. To account for subject withdrawal and dropouts due to adverse events, and non-compliance or personal reasons, 36 subjects were selected,

randomized and enrolled into the study. Hence a total of 36 subjects were enrolled in the study [45,46].

### Experimental design

The study was designed as open-labeled, balanced, randomized, two- treatment, two-sequence, two-period, single dose, crossover comparative bioequivalence study between test formulation (Acyclovir IR 800mg tablet) and reference product (Zovirax® 800mg IR tablet) with 7 days washout period in healthy, adult, Indian subjects under fasting and non-fasting conditions. Subjects were housed in the clinical facility before 12.00hours before drug administration to 24.00hours after drug administration in each period. Before enrollment, each subject was determined to be in good health basis of their vitals signs, physical examination and laboratory test results including serum chemistries, complete blood count with differential, 12-lead electrocardiogram (ECG) and X-ray chest. The alcohol breath test, urine drug of abuse test and urine pregnancy test (for female subjects only) were performed on the day of admission of each period of both fasting and non-fasting study. Thirty six subjects were selected and enrolled in each fasting and non-fasting study. All subjects were randomized as per the randomization schedule (two equal groups and assigned to one of the two sequences) generated in blocks using PROC PLAN such that the design is balanced using SAS software.

All subjects were fasted overnight atleast 10 hour before dosing for fasting study and fasted overnight atleast 10 hour before 30 minutes before intake of Food and Drug Administration (FDA) standard high-fat breakfast meal for non-fasting study. The standard high-fat breakfast meal consists of two slices of toast with two butter pads, 200 mL of whole milk, two butter egg omelet, one chicken cutlet and 100gms of french potato fries. The high-fat breakfast meal provided an estimated 66.2 g of carbohydrates (264.96 Calories and 26.78%), 37.13 g of protein (148.52 Calories and 15.01%), and 63.96 g of fat (575.64 Calories and 58.21%), and total 989.12 Calories, respectively [45,46]. Each subject received a single oral dose of test or reference products with 240 mL of water at room temperature in each period as per randomization schedule and continued fasting for 4 hours in both fasting and non-fasting study. Subjects were instructed not to chew or cross the drug at the time of administration. All subjects were dosed at the fixed time and were remained in sitting position for the first 2.00hours following drug administration. Drinking water was not allowed from one hour before dosing till two hour post-dose (except 240mL of water given for dosing) and before and after that subjects were allowed to ingest water *ad libitum*. Subjects were received identical, nutritionally balanced standard meals at 4.00, 9.00 and 13.00hours after dosing in each periods of both fasting and non-fasting study [45,46].

### Blood sample collection, Separation and storage

Blood sampling schedule was planned to provide an adequate estimation of C<sub>max</sub> and to cover the concentration-time curve long enough to provide a reliable estimate of extent of absorption. The blood samples were collected in pre-labeled vacutainer containing K<sub>2</sub>EDTA as anticoagulant. Blood samples of 5.0mL each was drawn at pre-dose (0.00 hr), 0.333, 0.667, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 16.00 and 24.00 hours after dosing. After collection of blood samples were centrifuge the samples at 4000 rpm for 10 minutes at 4°C. The plasma samples were separated and stored in a pre-labeled RIA vials at or below -20 ± 5°C till analysis [45,46].

### Method of sample analysis

A simple, specific, accurate and precise solid phase high performance liquid chromatographic method with Tandem Mass

Spectrometry-Waters Quattro Premier XE was developed and validated for the quantification of acyclovir in human plasma samples. The liquid chromatographic system consisted of Quattro Premier XE mass detector containing Mass Lynx version 4.2 chromatographic data system, auto sampler and column oven. Chromatographic analysis was performed using Hypersil GOLD C<sub>18</sub> column with 4.6 x 50 mm internal diameter in binary gradient mode and 5µm particle size with 0.5 mL/min flow rate [52-54].

The stock solution of acyclovir and internal standard (granciclovir) were prepared. The signal to noise ratio for system performance experiment by six consecutive injections at beginning of analytical batch was 29.2 for lower limit of quantitation (LLOQ) samples. The retention time of acyclovir and internal standard were approximately 1.19 and 1.42 minutes respectively. No significance interferences were observed in six different lots of human plasma, hemolysed plasma and lipemic plasma samples. The peaks were completely separated and there was no interference peak from endogenous substances in plasma that was co-eluted with acyclovir and internal standard. The Acyclovir lower limit of quantitation, signal to noise ratio and %CV of area ratio were 5.00ng/mL, 29.2 and 9.0% respectively. The overall chromatography run time was 2.21 minutes. Linearity was demonstrated by multiple analysis of spiked plasma sample containing acyclovir between 5.0 to 5000.0 ng/mL calibration ranges. The regression equation of acyclovir concentration over its peak area ratio was found to be  $y = 0.1383x + 0.012$ , where x is the concentration of acyclovir and Y is the respective peak area. The regression coefficient ( $r^2$ ) was 0.9998. A good linear relationship with the coefficient of determination ( $r^2$ ) of more than 0.999 was employed for determining of Acyclovir concentration in plasma. Back calculations were made from the calibration curves to determine Acyclovir of each calibration standard. The lower limit of quantification (LLOQ) was established at 0.5ng/mL with the coefficient of variation of 14.3% indicates the sensitivity of the method. Analyzed plasma Acyclovir concentrations below the quantification limit was defined as "zero" ng/mL. The % recovery was calculated from ratio of area of extracted to un-extracted samples at each level (six un-extracted samples each of low, medium and high quality control samples) were 105, 97.9 and 99.3 respectively. The mean % recovery for internal standard was 103. The high percentage of recovery of Acyclovir was found to be 101% indicates that the proposed method was highly accurate. The between-run % coefficient of variation ranged from 2.12 to 4.53 and within-run percentage of nominal value ranged from 95.31 to 101.15 respectively. The between-run % of nominal value ranged from 97.16 to 100.33 respectively [52-54].

### Pharmacokinetics and statistical analysis

Pharmacokinetic and statistical analysis for plasma concentrations vs time profile of acyclovir was performed in both fasting and non-fasting study on the data obtained from all subjects who have completed all the period of the study. Subjects for whom the pre-dose concentrations is greater than 5% of the  $C_{max}$  value for the subject in that period, subject data should be excluded for analysis. Pharmacokinetic primary parameters like  $C_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$  and secondary parameters like  $T_{max}$ , MRT (mean residence time),  $K_{el}$ ,  $t_{1/2}$  and  $AUC_{0-t}/AUC_{0-\infty}$  ratio were calculated using plasma concentrations vs. time profile data of both test and reference product in both fasting and non-fasting study by WinNonlin Professional Software program. All concentrations below the limit of quantification (LOQ) were set to "zero" for all pharmacokinetic and statistical analysis. The geometric mean ratios  $AUC_{0-t} / AUC_{0-\infty}$  were measured for the sampling schedule (sufficient number of samples to adequately describe the plasma concentration-

time profile were collected) around  $T_{max}$  to reliable estimate of peak and extent of exposure (i.e. not likely to lead to accumulation in the body) should be achieved by ratio  $AUC_{0-t} / AUC_{0-\infty} \geq 80\%$ , respectively [45,46,55].

Statistical analysis was performed for plasma concentration of pharmacokinetic parameters like  $C_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$  and  $T_{max}$  of both test and reference product in both fasting and non-fasting study by using SAS<sup>®</sup> Software program. Both un-transformed and ln-transformed pharmacokinetic parameters ( $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ ) and un-transformed pharmacokinetic parameters of  $T_{max}$  were analyzed using ANOVA model with the main effects of treatment, period and sequence as fixed effects and subjects nested within sequence as random effect. The  $T_{max}$  from test and reference product in both fasting and non-fasting study were compared using a non-parametric method (Wilcoxon Signed Rank Test at 5% level of significance). The effect test product in fasting and non-fasting conditions was compared to determine bioavailability with food (food effects) by student "t" test at 5% level of significance. The  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were compared using student "t" test at 5% level of significance under fasting and non-fasting conditions between test and reference product. Two one-sided "t" tests for bioequivalence of 90% confidence intervals for differences of least square mean (LSM) of ln-transformed test and reference product were calculated for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  for both fasting and non-fasting study. The power test (i.e. probability of detecting a 20% mean differences relative to the reference treatment LSM at the 5% significance levels using a t-test under null hypothesis of zero differences) was calculated for ln-transformed pharmacokinetic parameters of  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  for both fasting and non-fasting study. The bioequivalence criteria of ln-transformed parameters like  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were evaluated for test and reference product with bioequivalence acceptance range 80-125% for the 90% confidence intervals and compared in both fasting and non-fasting study. The dissolution results of test and reference product results and incidence of adverse events were compared between fasting and non-fasting study by student "t" test at 5% level of significance [45,46,55].

### Safety and tolerability assessment

The general clinical safety was assessed via clinical examination (physical and systemic examination) and vital signs (blood pressure, pulse rate, and oral temperature) were examined at screening, at the time of check-in, prior to administration of each study drug (0.0 hr), 1.00, 3.00, 6.00, 12.00 and 24.00 hours post dose, check-out, during the entire study period and the during follow-up visit of the study. Subjects were questioned for well being at the time of clinical examination, recording of sitting blood pressure and radial pulse and at the time of ambulatory blood sample collection. Alcohol breath tests, urine screen for drugs of abuse and urine pregnancy test (for female subject only) were performed at admission of each period for all subjects in both fasting and non-fasting study. Post study safety assessment (haematology and biochemical parameters- ALT, AST, Bilirubin, Creatinine and Urea) was assessed at the end of each period of the study (during 24.00 hours post dose blood sample). Adverse events were assessed for severity and relationship to treatment throughout the study and recorded by physician [45,46,55].

### Results

#### Subjects participant

Thirty six screened subjects were enrolled for each fasting and non-fasting study in good health conditions. The demographic profiles all 36 subjects participated into each acyclovir fasting and non-fasting

study were shown in Table 1. Total 36 subjects enrolled into the fasting study and all were completed the both periods of the study. Total 36 subjects were enrolled into the non-fasting study and 34 subjects were completed the both periods of the study. Two subjects were dropped out in non-fasting study due to their personal family reasons. Weight, height, BMI, age, smoking and tobacco consumption of the each subject were recorded during screening. Weight and heights of the subjects were within the limit of the normal range according to normal values for the BMI ranges from 18.10 to 24.94 Kg/m<sup>2</sup> for fasting study and 18.25 to 23.94 Kg/m<sup>2</sup> for non-fasting study. Age of the subjects was within range of 20 to 42 years of both fasting and non-fasting study. There were 13.88% and 2.78% tobacco users ( $\leq$  2-4 packets per day) screened and enrolled into the fasting and non-fasting study. All enrolled subjects were discontinued from smoking and tobacco consumptions 48 hours before initiation of study and during the study period except two drop-out subjects. All subjects were dosed test and reference product as per the randomized schedule in both fasting and non-fasting study and compliance for dosing was assessed by a thorough check of the oral cavity using torch immediately after dosing.

### In vitro dissolution test

**Acyclovir tablet formulation:** Acyclovir is an ampholytic (both weak acid and basic groups) drug and solubility of acyclovir is dependent on its ionization constant and the pH of the environment. The pKa of acyclovir was reported as 2.27 and 9.04 at 37°C [24,36]. The pH-solubility in deionized water at 37°C showing the solubility to vary slightly with pH, with a lowest solubility of 2.4 mg/mL at pH 5.5 at 37°C [50]. The solubility (D:S) ratio of 250mL evaluated in different media (except water) were higher than 3.5 mg/mL and lower value 2.4 mg/mL in aqueous media at 37°C [1,50]. The above dissolution profile and disintegration test results shows that acyclovir was ionized form in the upper GI tract environment and was more soluble in more acidic pH conditions [1,50].

The physical properties like tablet hardness, weight values obtained and acyclovir content uniformity in both test and reference product were consistent and within the acceptance range [50]. The disintegration test results demonstrated that acyclovir tablets held different release kinetics compared to reference product as anticipated.

The release profile of acyclovir test and reference product in SGF<sub>sp</sub> and FeSSIF medium were shown in Figures 1 to 2 and comparative release profile in Figure 3, respectively. The dissolution behaviors of acyclovir test and reference product dissolved quicker in 0.1N HCL, FeSSIF and SGF<sub>sp</sub> medium were 90.25%, 91.45% and 92.16% in 30 minutes, respectively, which was more than or equal to 85% of drug substance is released within 30 minutes [50,51]. The dissolution results exhibited that the release profiles of test and tablet formulation

acquired were similar across the different dissolution media (both fasted and non-fasted state) indicating their robustness of release characteristics in the different conditions extant in the upper GI tract milieu. The similarity of dissolution patterns obtained between FeSSIF and SGF<sub>sp</sub> medium were construed by the physicochemical properties of acyclovir itself. The aqueous solubility of acyclovir has a minimum value of 3.5 mg/ml across the pH range in the GI tract was considered as highly soluble at lower pH values studied [1]. The dissolution profile of acyclovir test and reference product in FeSSIF and SGF<sub>sp</sub> medium according to “model independent approach”, the difference factors (f1) was 8.57 and 7.56 and similarity factor (f2) was 86.98 and 88.63, respectively indicating the mutual release profile pf the drug.

**Commercially available Acyclovir 800mg IR tablets:** The dissolution behaviors of all commercially available acyclovir tablet products dissolved quicker in 0.1N HCL, SGF<sub>sp</sub>, FeSSIF and SIF<sub>sp</sub> medium, which was more than 85% of drug substance is released within 30 minutes [48-50]. The solubility was possibly because of the component of micro-crystalline cellulose (Avicel<sup>®</sup> PH101) composed in the tablet formulations as well as the physical appearance of tablets facilitating their rapidly dissolving properties [24,36].

### Subject sample analysis

The acyclovir in human plasma was quantified using validated LC/MS/MS liquid chromatography method with Mass spectrometer detection mode. Total 36 subject samples for fasting study and 34 subject samples for non-fasting study plasma concentrations were analyzed. The analysis of subject samples was done by using a calibration curve with quality control samples, distributed throughout each batch. Samples with acyclovir concentrations greater than the upper limit of validated range of the assay were diluted with the appropriate drug-free biological fluid and re-assayed. The stability of acyclovir contents in plasma analyzed in the series of concentrations (six stability samples of each lower and higher quality control samples were kept frozen in vials at -20 ± 5°C) for 1, 2 and 4 months were assessed with freshly processed calibration and comparison samples (six samples each of lower and higher quality control samples). The % acyclovir change found for lower and higher quality control samples were 2.45 and 0.83 respectively. The results suggested that acyclovir was stable in human plasma at -20 ± 5°C for atleast 4 months and expected to be more stable at -70±5°C.

### Pharmacokinetic and statistical analysis

The pharmacokinetic and statistical parameters were analyzed from 36 subject's data sets for fasting study and 34 subject's data sets for non-fasting study were completed all the periods of the study. Unbalanced sequences were considered for calculations of two dropout

Study Type	Gender, N	Age <sup>1</sup>		Body weight <sup>1</sup> (Kg)		Height <sup>1</sup> (cm)		BMI <sup>1</sup> (Kg/m <sup>2</sup> )		Tobacco User <sup>2</sup>
		Mean± SD	Range	Mean± SD	Range	Mean± SD	Range	Mean± SD	Range	Yes/No
Fasting (N=36)	Male, 18	26.78 ± 5.17	38-20	58.41 ± 7.83	73.00-46.50	168.45 ± 6.06	179.00-159.00	20.57 ± 2.47	24.94-18.10	No, 15 (83.33%) Yes, 3 (16.67%)
	Female, 18	34.09 ± 6.21	42-22	51.84 ± 5.73	61.40-43.80	152.16 ± 6.69	165.40-142.00	22.36 ± 1.30	24.81-19.74	No, 16 (88.89%) Yes, 2 (11.11%)
Non-fasting N=36)	Male, 18	29.94 ± 7.57	44-20	54.51 ± 8.43	75.00-45.00	164.66 ± 6.35	179.20-157.00	20.01 ± 1.91	23.94-18.25	No, 17 (94.44%) Yes, 1 (5.56%)
	Female, 18	31.44 ± 5.54	42-22	49.80 ± 4.50	58.00-41.10	152.92 ± 6.09	165.00-143.00	21.30 ± 1.49	23.24-18.27	No, 18 (100.00%) Yes, 0 (0.00%)

SD: Standard Deviation; N= Number of Subjects

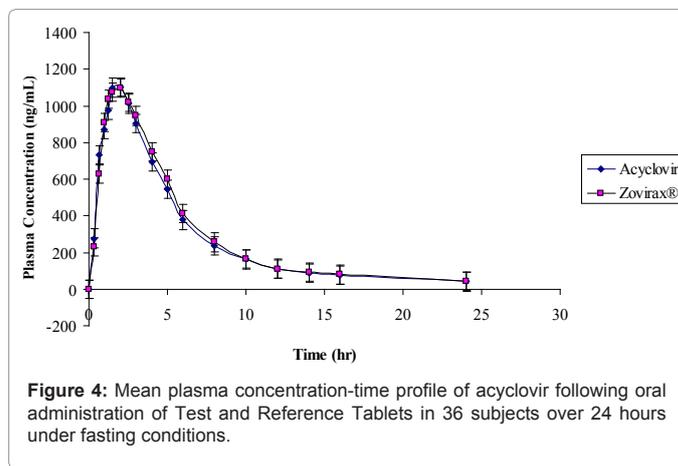
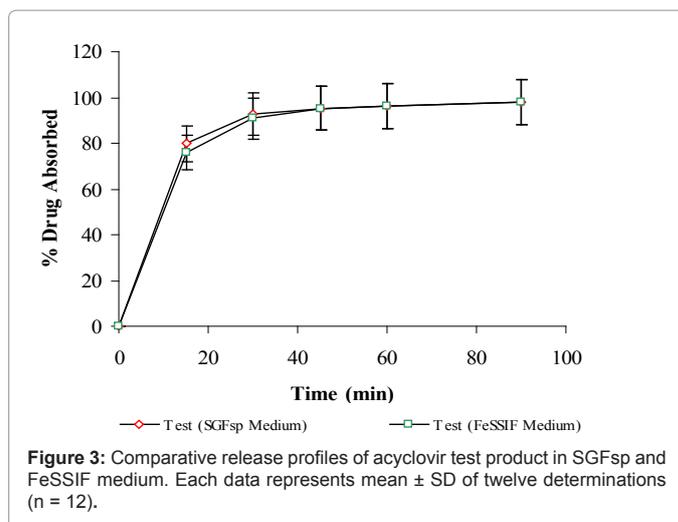
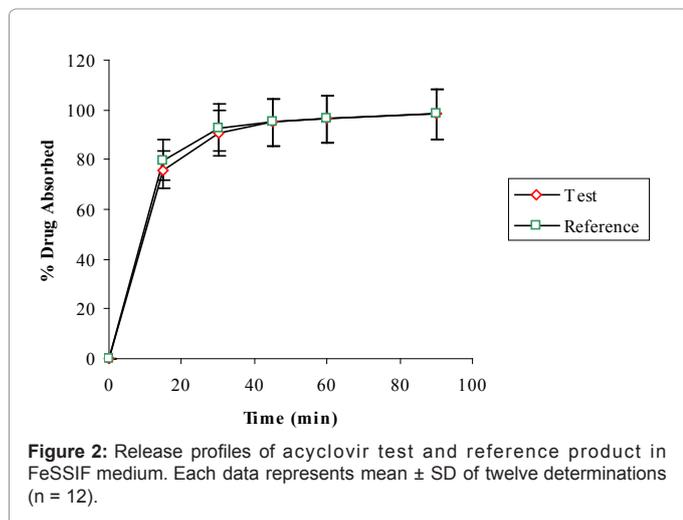
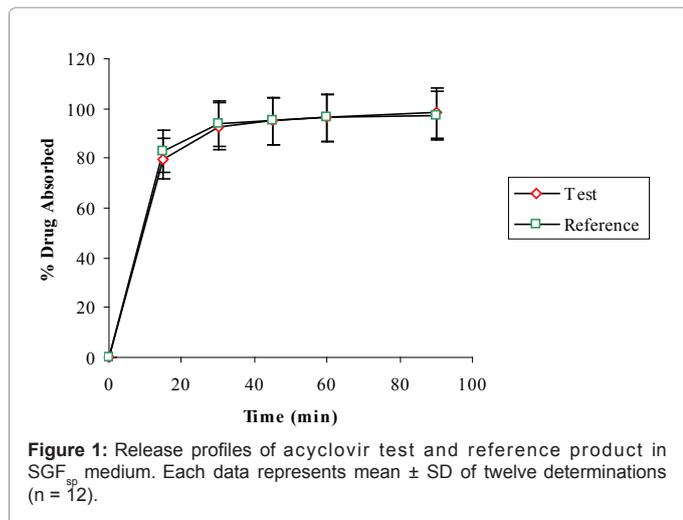
<sup>1</sup>Determined at Screening

<sup>2</sup>Defined as current tobacco user (consuming tobacco  $\leq$  2-4 packets per day since 30 days of screening)

**Table 1:** Demographic profiles of subjects participated into acyclovir 800mg IR tablet under fasting and non-fasting study.

subjects with the same sequence in non-fasting study. The individual and comparative mean pharmacokinetic parameters estimated for the test and reference product for both fasting and non-fasting study were summarized in Table 2 and Figures 4 to 6 respectively.

The rate of absorption of acyclovir immediate release test and reference products were analyzed by pharmacokinetic parameters  $C_{max}$ ,  $T_{max}$  and MRT for both fasting and non-fasting study. The mean  $\pm$  SD  $C_{max}$  for test and reference products were  $1158.124 \pm 518.249$  ng/mL and  $1227.654 \pm 653.981$  ng/mL under fasting condition and  $1387.719 \pm 554.165$  ng/mL and  $1409.831 \pm 517.194$  ng/mL under non-fasting conditions, respectively. The mean  $\pm$  SD  $T_{max}$  for test and reference products were  $1.920 \pm 0.878$  hours and  $1.976 \pm 1.197$  hours under fasting condition, and  $2.729 \pm 1.177$  hours and  $2.771 \pm 1.130$  hours under non-fasting conditions, respectively. The mean  $\pm$  SD MRT for test and reference products were  $5.664 \pm 0.667$  hours and  $5.734 \pm 1.273$  hours under fasting condition and  $5.699 \pm 1.074$  hours and  $5.941 \pm 1.012$  hours under non-fasting conditions, respectively. The extent of absorption of acyclovir immediate release test and reference products were analyzed by pharmacokinetic parameters  $AUC_{0-t}$  and  $AUC_{0-\infty}$  for both fasting and non-fasting study. The mean  $\pm$  SD  $AUC_{0-t}$  for test and reference products were  $5417.395 \pm 2681.840$  ng.hr/mL and  $5562.313 \pm 2945.785$  ng.hr/mL under fasting condition and  $6285.005 \pm 2155.759$



ng.hr/mL and  $6793.396 \pm 2604.046$  ng.hr/mL under non-fasting conditions, respectively. The mean  $\pm$  SD  $AUC_{0-\infty}$  for test and reference products were  $5789.848 \pm 2746.581$  ng.hr/mL and  $5890.051 \pm 3016.390$  ng.hr/mL under fasting condition and  $6523.251 \pm 2219.038$  ng.hr/mL and  $7057.526 \pm 2670.944$  ng.hr/mL under non-fasting condition, respectively. Other pharmacokinetic parameters like  $t_{1/2}$ ,  $K_{el}$  and  $AUC_{0-t}/AUC_{0-\infty}$  measured were not required for comparative statistical analysis. The mean  $\pm$  SD  $t_{1/2}$  for test and reference products were  $6.181 \pm 4.233$  hours and  $6.410 \pm 3.947$  hours under fasting condition and  $5.179 \pm 1.982$  hours and  $5.389 \pm 3.372$  hours under non-fasting conditions, respectively. The mean  $\pm$  SD  $K_{el}$  for test and reference products were  $0.121 \pm 0.048$  hour<sup>-1</sup> and  $0.134 \pm 0.050$  hour<sup>-1</sup> under fasting condition and  $0.155 \pm 0.067$  hour<sup>-1</sup> and  $0.155 \pm 0.055$  hour<sup>-1</sup> under non-fasting conditions, respectively. There were no statistical significance ( $p > 0.05$ ) difference of individual and comparative pharmacokinetic parameters  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  between test and reference formulations were observed in both fasting and non-fasting study. These results indicated that food dose not affects the rate and extent of acyclovir absorption in systemic circulation. The mean  $\pm$  SD  $AUC_{0-t} / AUC_{0-\infty}$  ratio for test and reference products were  $92.740 \pm 6.027$  and  $94.062 \pm 4.935$  hours under fasting condition and  $96.270 \pm 2.140$  and  $95.980 \pm 4.850$  under non-fasting conditions shows that the time point of sample collection were near  $T_{max}$  and reliable to estimate the rate and extent of acyclovir absorption [55].

Pharmacokinetic Parameters	Geometric mean $\pm$ SD (CV%)			
	Fasting Condition*		Non-Fasting Condition**	
	Test (A)	Reference (B)	Test (A)	Reference (B)
C <sub>max</sub> (ng/mL)	1158.124 $\pm$ 518.249 (44.75)	1227.654 $\pm$ 653.981 (53.27)	1387.719 $\pm$ 554.165 (39.93)	1409.831 $\pm$ 517.194 (36.69)
AUC <sub>0-t</sub> (ng.hr/mL)	5417.395 $\pm$ 2681.840 (49.50)	5562.313 $\pm$ 2945.785 (52.96)	6285.005 $\pm$ 2155.759 (34.30)	6793.396 $\pm$ 2604.046 (38.33)
AUC <sub>0-∞</sub> (ng.hr/mL)	5789.848 $\pm$ 2746.581 (47.44)	5890.051 $\pm$ 3016.390 (51.21)	6523.251 $\pm$ 2219.038 (34.02)	7057.526 $\pm$ 2670.944 (37.85)
T <sub>max</sub> (hr)	1.920 $\pm$ 0.878 (45.70)	1.976 $\pm$ 1.197 (60.58)	2.729 $\pm$ 1.177 (43.13)	2.771 $\pm$ 1.130 (40.78)
Kel (1/hr)	0.121 $\pm$ 0.048 (39.89)	0.134 $\pm$ 0.050 (37.78)	0.155 $\pm$ 0.067 (43.26)	0.155 $\pm$ 0.055 (35.25)
T <sub>1/2</sub> (hr)	6.181 $\pm$ 4.233 (58.95)	6.410 $\pm$ 3.947 (61.57)	5.179 $\pm$ 1.982 (38.26)	5.389 $\pm$ 3.372 (62.56)
MRT (hr)	5.664 $\pm$ 0.677 (11.95)	5.734 $\pm$ 1.273 (22.20)	5.699 $\pm$ 1.074 (18.85)	5.941 $\pm$ 1.012 (17.03)
AUC <sub>0-t</sub> /AUC <sub>0-∞</sub>	92.740 $\pm$ 6.027 (6.50)	94.062 $\pm$ 4.935 (5.25)	96.270 $\pm$ 2.140 (2.22)	95.980 $\pm$ 4.850 (5.05)

\*N= 36 for fasting study and \*\* N=34 for non-fasting study; CV = Coefficient of Variance

**Table 2:** Mean pharmacokinetic parameters of acyclovir following oral administration of Test and Reference products over 24 hr under fasting and non-fasting conditions.

Parameters	Product	Fasting study			Non-fasting study		
		C <sub>max</sub> (ng/mL)	AUC <sub>0-t</sub> (ng.h/mL)	AUC <sub>0-∞</sub> (ng.h/mL)	C <sub>max</sub> (ng/mL)	AUC <sub>0-t</sub> (ng.h/mL)	AUC <sub>0-∞</sub> (ng.h/mL)
Geometric Mean	Test (A)	1158.124	5417.395	5789.848	1387.719	6285.006	6523.251
	Reference (B)	1227.654	5562.313	5890.051	1409.831	6793.396	7057.526
Least Square Mean (LSM)	Test (A)	1100.218	5146.525	5500.356	1339.339	5970.755	6197.088
	Reference (B)	1166.271	5284.197	5595.548	1318.333	6453.726	6704.65
LSM Ration A/B %		94.34	97.39	98.30	98.43	92.52	92.43
90% Confidence Interval : A/B							
Lower Limit		97.82	87.22	86.45	96.28	86.39	86.29
Upper Limit		102.01	113.34	100.78	110.16	102.15	101.42
P – value (ANOVA)							
Period		0.7251	0.7701	0.1303	0.4266	0.6594	0.4148
Formulation		0.9077	0.9981	0.7624	0.5238	0.7722	0.8947
Sequence		0.0562	0.0217	0.0142	0.9900	0.4586	0.0567
Intra-subject Variability: (CV%)		14.10	16.04	14.7	18.52	17.02	16.39
Power (%)		99.99	98.73	99.46	98.96	99.54	99.69
Untransformed T <sub>max</sub> (p-value) (Wilcoxon signed rank test)			0.9473			0.9727	

Note: A= Test Product and B=Reference Product; CV = Coefficient of Variance

**Table 3:** Summary statistics of acyclovir in subjects under fasting and non-fasting conditions.

The geometric least square means, ratio (A/B)% of geometric least square mean and 90% confidence interval (90% CI) for the ratio (A/B)% based on root mean square error obtained from ANOVA for the pharmacokinetic parameters C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> for fasting and non-fasting study were summarized in Table III. Period and formulation effects for ln-transformed pharmacokinetic parameter C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> and sequence effects of C<sub>max</sub> were statistically insignificant for both fasting and non-fasting study. Sequence (carryover) effects for ln-transformed pharmacokinetic parameter AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were statistically significant (p<0.05) for fasting study and insignificant for non-fasting study. The sequence effects were acceptable because of single dose, healthy subjects with adequate washout period without any detectable drugs detected in pre-dose analyzed samples.

The ratio of geometric least square means for the (A/B) of C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were 94.34%, 97.39% and 98.30% under fasting condition and 98.43%, 92.52% and 92.43% under non-fasting conditions, respectively. The 90% confidence interval for the (A/B) of C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were 97.82% - 102.01%, 87.22% - 113.34% and 86.45% - 100.78% under fasting condition and 96.28% - 110.16%, 86.39% - 102.15% and 86.29% - 101.42% under non-fasting conditions, respectively. The intra-subject variability for ln-transformed data of the C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were 14.10%, 16.04% and 14.7% under fasting condition and 18.52%, 17.02% and 16.39% under non-fasting conditions, respectively. The power of the test for the ln-transformed

pharmacokinetic parameters C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were found to be 99.99%, 98.73% and 99.46% under fasting condition and 98.96%, 99.54% and 99.69% under non-fasting conditions, respectively. The 90% confidence Interval of the medium of the differences of T<sub>max</sub> for both test and reference products were insignificant (p>0.05) changes in both fasting and non-fasting study of acyclovir and were comparable between the study. The sequence (carry-over) effects for ln-transformed pharmacokinetic parameter AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were statistically significant (p<0.05) for fasting study (see Table III). The result shows that the point estimate and upper 90% CIs of C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> are the acceptable range in both fasting and non-fasting study and meeting the predetermined criteria for bioequivalence ranges of 80-125% suggested by the USFDA bioequivalence guideline.

### Safety and tolerability

Few subjects taking both test and reference products were noted mild adverse events in both fasting and non-fasting study. The comparative safety and tolerability profile of acyclovir following oral administration of test and reference products over 24 hr under fasting and non-fasting conditions were summarized in Table 4. Total 07 subjects of the safety population (N=36) reported a total 26 adverse events (test 11 and reference 15) during the fasting study, 03 of these subjects after treatment with test product and 04 after reference products. Total 06 subjects of the safety population (N=34) reported a total 25 adverse events (test 13 and reference 12) during the non-fasting study, 03 of these subjects after treatment with test product and 03

Adverse events	Severity <sup>1</sup>	Relationship to Drug <sup>2</sup>	Fasting Study, N=36			Non-fasting Study, N=34 <sup>3</sup>		
			Reported incidence		Total incidence	Reported incidence		Total incidence
			Test (Acyclovir)	Reference (Zovirax®)		Test (Acyclovir)	Reference (Zovirax®)	
Itching	1	2	1(2.78%)	1(2.78%)	2 (2.78%)	1(2.86%)	--	1 (1.43%)
Nausea	1	3	2 (5.56%)	3 (8.33%)	5 (6.94%)	2 (5.71%)	2 (5.71%)	4 (5.71%)
Headache	1	3	1(2.78%)	2 (5.56%)	3 (4.17%)	--	1(2.86%)	1 (1.43%)
Dizziness	1	3	--	1(2.78%)	1(1.39%)	1(2.86%)	1(2.86%)	2 (2.86%)
Sleepiness	1	3	--	1(2.78%)	1(1.39%)	1(2.86%)	1(2.86%)	2 (2.86%)
↑ ALT	1	2	2 (5.56%)	1(2.78%)	3 (4.17%)	1(2.86%)	2 (5.71%)	3 (4.29%)
↑ AST	1	2	1(2.78%)	2 (5.56%)	3 (4.17%)	2 (5.71%)	1(2.86%)	3 (4.29%)
↑BP	1	2	3 (8.33%)	2 (5.56%)	5 (6.94%)	4 (11.43%)	3 (8.57%)	7 (10.00%)
↓BP	1	2	1(2.78%)	2 (5.56%)	3 (4.17%)	1(2.86%)	1(2.86%)	2 (2.86%)
<b>Total</b>			<b>11 (31.43%)</b>	<b>15 (41.67%)</b>	<b>26 (36.11%)</b>	<b>13 (37.14%)</b>	<b>12 (34.29%)</b>	<b>25 (35.71%)</b>

<sup>1</sup>Severity of Adverse event: 1-Mild; 2-Moderate, 3-Severe

<sup>2</sup>Relationship to Drug: 1-Unrelated; 2-Remote; 3-Possible; 4-Probable; 5-Define

<sup>3</sup>Total 34 subjects in each test and reference formulation

Abbreviations: ↑= Increase; ↓= Decrease; ALT: Alanine transaminase; AST= Aspartate transaminase; BP= Blood Pressure

N%= Number of subjects reporting AE / number of subjects dosed with respective study drug (x 100). Total N% = Number of subjects that reported at least one AE / number of subjects dosed with respective study drug (x 100)

**Table 4:** Comparative safety and tolerability profile of acyclovir following oral administration of Test and Reference products over 24 hr under fasting and non-fasting conditions.

after reference products. Two subjects were dropped out for personal reasons in non-fasting study. The most common adverse events like itching, nausea, headache, dizziness, and sleepiness reported were mild in severity and possible relation to the drug. The abnormal vital signs and laboratory parameters were mild in severity and remotely relationship to drug. There were increased in post safety laboratory parameters like AST and ALT observed in 03 subjects (one test and two reference product) in fasting and 03 subjects (two test and one reference product) in non-fasting study and all laboratory values were found within normal clinical range after follow-up visits. There were abnormalities in vital signs (blood pressure) observed in 04 subjects (two test and two reference product) in fasting and 04 subjects (two test and two reference product) in non-fasting study during the study and were found normal during vital signs taken during check-out in each periods. There were no premature withdrawals, replacements or death during the study. No unexpected incidents that could have influenced the outcome of the study occurred. No subjects had any serious adverse events or withdrawn from the study because of adverse event. There were no clinical significant changes in vital signs, clinical laboratory variables, ECG parameters or physical examination findings in both fasting and non-fasting study.

## Discussion

The results of this study demonstrates that a single dose of acyclovir 800mg immediate release tablet in fasting conditions were similar to total exposure (as measured by AUC), safety and tolerability were compared to non-fasting study throughout a 24 hours period. However, the peak plasma concentration ( $C_{max}$ ) and the time to peak plasma concentrations ( $T_{max}$ ) were higher in test product under non-fasting conditions compared to fasting conditions. The results (see Figure 1 to 3) suggested that test product was more rapid absorbed in under fasting conditions with insignificance difference ( $p>0.05$ ) compared to the non-fasting conditions and were consistent with the results of the dissolution testing and food does not affect the rate of absorption of acyclovir into systemic circulation.

## In vitro dissolution test

In terms of the BCS concept, analogous to BCS Class I, it was expected that immediate release products containing BCS Class III

compounds (highly soluble-poorly permeable) would behave similarly *in vivo*, provided that dissolution from the drug product was rapid under all physiological pH conditions and that the excipients used exert no effect either on upper gastrointestinal (GI) motility or compound permeability [49,59,60]. In this study, acyclovir tablet formulations were conceived to cover release characteristics ranging from “rapid enough to facilitate absorption” through to “slow enough to retard or even possibly reduce absorption”. According to the ‘Model Independent Approach’ the the release profiles of acyclovir test formulation and reference product different from each other were  $f1<15.00$  and  $f2>50.00$ , which indicates the mutual similarity of the compared release profiles due to the physicochemical properties of acyclovir itself [61]. The results of this study clearly revealed that a dissolution specification for acyclovir, a BCS Class III compound, of 85% drug release in 30 minutes under BCS-conform conditions would result in comparable pharmacokinetic parameters, indicating bioequivalency of acyclovir products and permeability-limited absorption [51]. The solubility results demonstrated that acyclovir was a highly soluble in acidic medium pH conditions. The ten acyclovir IR products randomly selected from the different zone of the India exhibited rapid release behavior, i.e., more than 85% of the active ingredient was released within 30 minutes, indicating that permeability, rather than dissolution properties of the acyclovir products and was the rate-determining step to overall drug absorption [51].

## Acceptance range peak plasma concentration ( $C_{max}$ )

The results illustrating that the pharmacokinetic parameters  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  of the test formulation varies substantially from 2-6% compared to reference product in both fasting and non-fasting study which were statistical insignificant ( $p>0.05$ ). The  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  values reported for the present study was proved to be within the range 80-125% in both fasting and non-fasting study and were comparable with other study conducted [30,33]. The  $AUC_{0-t}/AUC_{0-\infty}$  ratio more than 80% indicates that sufficient number samples were collected for adequate description of plasma-time profile concentrations of acyclovir test formulation [51]. The dissolution test result shown that acyclovir test formulation was rapidly dissolved *in vitro* fasting and non-fasting dissolution medium resulting in > 85% drug release in 30 minutes. This observation was compatible with the

faster ( $T_{max}$ ) and higher peak plasma concentrations ( $C_{max}$ ) observed in the present cross-over study in healthy subjects in both fasting and non-fasting study. The values obtained were not trespass the pre-defined acceptance range for  $C_{max}$  as outlines above [30,33].

### Rate and extent of absorption ( $C_{max}$ , $T_{max}$ , MRT and AUC)

The acyclovir because of its high hydrophilic nature, was absorbed in the small intestine in a passive, variable and incomplete manner [30]. Indeed, acyclovir has been replaced by its prodrug, valacyclovir, as a means of increasing systemic availability [26]. There are also possible to increase the oral absorption of acyclovir by improving the formulation [32,33,54].

The results from both fasting and non-fasting study, shows that the pharmacokinetic parameters  $C_{max}$ ,  $T_{max}$ , MRT,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  of the test and reference products varies from 14-22% and were statistically insignificant ( $p>0.05$ ) and comparable. The reason of delaying rate and extent of acyclovir absorptions were due to the absorption window or a gradient in permeability of the gut wall to the drug (with decreasing permeability in distal regions) [17-20]. The foods that accelerate gut motility was significantly delaying the contact time of the acyclovir with its absorption sites in the gut at which permeability was favorable and therefore increase in bioavailability of the acyclovir in non-fasting study compared to fasting study [32,35,38,58]. Therefore, higher bioavailability from non-fasting study compared with fasting study has been reported for acyclovir [29,30,33], although mean residence time and elimination half-life values for both fasting and non-fasting studies were statistically insignificant ( $p>0.05$ ), comparable and provides a good indication of the similar time spent for a drug to be removed from the body [28,31] The sequence (carry-over) effects for

In-transformed pharmacokinetic parameter  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were statistically significant ( $p<0.05$ ) for fasting study due to the acceptable of a single dose healthy subjects with an adequate washout period and without any level of detectable drug in all analysed subjects pre-dose samples [62].

The present study in healthy volunteers showed that the test tablet formulation of acyclovir produces lower plasma concentrations than the reference tablet. The point estimate and upper 90% CIs of  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were within the acceptable range of 80 and 125 % in both fasting and non-fasting conditions. The study results revealed that the two formulations of acyclovir were similar in pharmacokinetic characteristics among these healthy Indian volunteers. The pharmacokinetic values for acyclovir from reference product (Zovirax® 800mg IR tablets) obtained in this study were in good agreement with those from other studies [29,30,33].

### Safety and tolerability assessment

Acyclovir has shown occasional reports of acute, usually reversible, renal failure and neurotoxicity associated with highest dose oral pharmaceutical formulation in a number of clinical studies [37,42], but these were more often with intravenous administration, usually patients with high peak plasma acyclovir concentrations. Acyclovir was well tolerated whether administration by ocular, topical, oral or intravenous routes [41]. The incidences of most adverse events, such as gastrointestinal symptoms, such as nausea, vomiting, diarrhoea, stomach pain, rash and headache, occurring in fewer than 5% of patients during oral acyclovir therapy was similar to that seen in patients receiving placebo [41]. There were few adverse events were reported during the fasting and non-fasting study. The most frequently reported adverse events after acyclovir treatment were itching, nausea, headache, dizziness and sleepiness adverse incidence were mild in severity and appeared possible to be drug-related (see Table 4) [28,41]. The abnormal vital signs (blood pressure) and laboratory biochemical parameters (ALT and AST) were mild in severity and remotely related to the study medication. Post study safety analysis reports showed ALT and AST abnormal laboratory values were found within clinical acceptance ranges after follow-up visit. All were mild in intensity and resolved without sequel. In few incidences in which increase and decrease the blood pressure during study and found resolved during vital signs at the time of check-out of subjects. However, no significant changes in vital signs, clinical laboratory variables, ECG parameters or physical examination findings were observed in both fasting and non-fasting study. All adverse reported for acyclovir were consistent with similar events in subjects treated with fasting and non-fasting condition. As far as this study is concerned, both the test and the reference formulations were safe and well tolerated.

### Conclusion

For the purpose of establishing fasting and food effects, proof of pharmacokinetic, safety and tolerability to reference product was mandatory for newly developed generic acyclovir tablets. The results of the present study suggest that the acyclovir test product (800mg IR tablet) was comparable to the reference product (Zovirax® 800mg Immediate release tablet) based on the rate and extent of absorption in both fasting and non-fasting study. The minor differences were the food probably affect the contact time of acyclovir with the sites of absorption in the gut. The both test and reference products were well tolerated and all subjects (except two who were dropout from study) who started the study continued to the end and were discharge in good health. Acyclovir was found safe and well tolerated by all analyzed

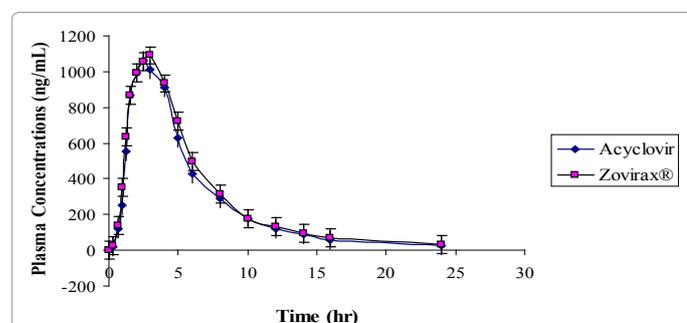


Figure 5: Mean plasma concentration-time profile of acyclovir following oral administration of Test and Reference Tablets in 34 subjects over 24 hours under non-fasting conditions.

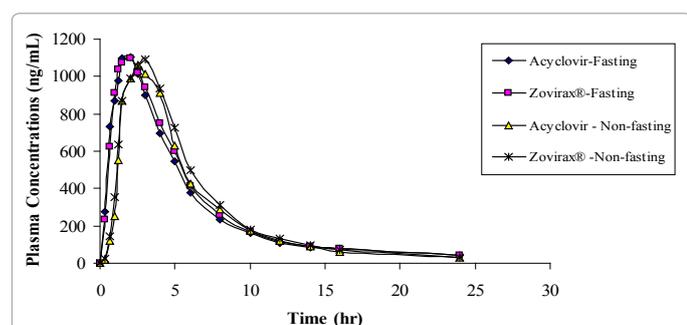


Figure 6: Comparative mean plasma concentration-time profile of acyclovir following oral administration of Test and Reference Tablets over 24 hours under fasting and non-fasting conditions.

subjects and no unexpected incident that was influenced the outcome of the study occurred.

#### Acknowledgements

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