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ASSESSMENT OF FAMOTIDINE BIOAVAILABILITY IN LOCAL POPULATION OF BAHAWALPUR, PAKISTAN: A STEP TOWARDS EFFECTIVE USE OF THE DRUG

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ABSTRACT

Plasma profile of the Famotidine, cited in various reports is according to the data collected from different regions of the world except local areas of Pakistan. To date it's hard to find any study carrying out evaluation of plasma concentration of Famotidine in Pakistani population. Current study was carried out to investigate the Pharmacokinetic parameters of the said drug in local population. Bioavailability study of famotidine in Pakistan for the first time was conducted. In this study Pepsidine[®] by Merck was used. Single blind, Cross over, overnight fasting study with a washout period of seven days was conducted. All the necessary pharmacokinetic parameters were calculated. C_{max} was found to be 1.088 $\mu\text{g/ml}$ to 1.950 $\mu\text{g/ml}$ with mean $1.559 \pm 0.077 \mu\text{g/ml}$. T_{max} was from 1.5 - 2.0 hours with mean 1.667 ± 0.071 hours. Time (MRT) was found to be from 2.798 – 4.911 hours with mean 3.745 ± 0.159 hours. AUC $0-\infty$ was from 8.0412 to 15.3812 $\mu\text{g.h/ml}$ with mean $11.480 \pm 0.607 \mu\text{g.h/ml}$. AUMC $0-\infty$ was from 24.027 – 70.166 $\mu\text{g.h}^2/\text{ml}$ with mean $43.869 \pm 3.979 \mu\text{g.h}^2/\text{ml}$. VD was from 10.904-16.225 L/Kg with mean 13.198 ± 0.418 L/Kg. V_{ss} was from 0.570-1.665 L/Kg with mean 1.00 ± 0.094 L/Kg which is a little bit higher plasma half-life was from 1.939 -3.403 hours with mean $1.979 \pm .110$ hours. K_e was 0.204-0.357 with mean 0.394 ± 0.012 . Cl (ml/h/kg) values were 2.601-4.974 ml/h/Kg with mean value of 3.598 ± 0.199 ml/h/Kg. From results it is evident that Famotidine the reported pharmacokinetic parameters are approximately same as were recorded in the current study.

Keywords: Famotidine, H_2 -receptor antagonist, GERD, Peptic ulcer, Pharmacokinetic

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INTRODUCTION

Famotidine was introduced few years back in 1986. It is N' - (aminosulfonyl)-3-[[[2-[(diaminomethylene) amino]-4-thiazolyl] methyl] thio] propanimidamide [1]. It is highly specific to H_2 receptors, with little activity at H_1 receptors or anticholinergic receptors. As common property of H_2 -receptor antagonists is that these are weak inhibitors of hepatic cyto-chrome p450 mixed function oxidase system [2]. After oral dosing, acid suppression occurs within 1 hour while maximum effect occurs within 1 to 3 hours [3]. Effective acid suppression in children lasts from 5 to 9 hours. Volume of distribution in children varies from 1.4 to 2.4 L/kg. Elimination half-life ranges from 2.2 to 3.3 hours. Clearance values range from 0.3-0.48

L/hr/kg [4]. Excretion is mainly by renal tubules [5].

MATERIALS AND METHOD

Famotidine (Cadila Healthcare Limited Gujarat India Batch Number FM/005/4119, Methanol (Merck, Germany), Double Distilled Water (The Islamia University of Bahawalpur), O-Phosphoric Acid, Sodium Hydroxide, Acetonitrile (Merck, Germany), Pepsidine[®]

Study Design

Twelve healthy human volunteers were included in the study for the evaluation of plasma concentration of famotidine. Written consent was obtained from each subject prior to the conductance of study.

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Inclusion Criteria

Subjects of age from 18 to 33 years were included with a weight range of 50 – 70 Kg and with good health and having no previous anomaly in their medical history. Complete physical, blood and urine analysis was done prior to commencement of study.

Exclusion Criteria

Subjects were excluded from the study if any volunteer had a history of kidney disease or decreased creatinine clearance (less than 50ml/min), hepatic or cardiovascular diseases, or a hematocrit of <36% at screening. They also were excluded if any condition was known to interfere with the absorption of drug. Volunteers with abnormal findings upon medical history, physical examinations, and screening tests, with positive human immunodeficiency virus (HIV) serology test, AIDS, with a history of hypersensitivity to Famotidine or any other H₂-Receptor antagonist, weight was >130% of ideal body weight or had donated blood within 30 days prior to the study were also excluded. Volunteers With limited mental capacity to the extent that subject was unable to provide the legal consent and information regarding the side effects or tolerance to the study drug was also excluded [7]

Clinical Tests

All the clinical tests including LFTs and RFTs were performed which are prerequisites prior to the conduction of pharmacokinetic studies. So only healthy volunteers free from serious, contagious and chronic diseases would be included in the study. And during the study all the safety profile of the volunteers was kept maintained.

Experimental Design

Experimental work was performed at the Department of Pharmacy, the Islamia University of Bahawalpur. Twelve Healthy Human subjects participated in the study. These volunteers received Pepsidine®, 40 mg, and a single dose after overnight fasting. 40 mg dose was administered on an empty stomach at 0800 AM. The volunteers were not allowed to eat anything at the night and two hours after administration of drug. The subjects were allowed to drink water. Each subject was provided with a standard breakfast two hours after the dosing. They were also provided with standard lunch and evening refreshment.

Sample Collection Schedule

A 20-gauge venous catheter was inserted into the forearm of each subject for the collection of blood samples and 10 blood samples each of 5 ml blood were collected at 0.0, 0.5, 1.0, 1.50, 2.0, 3.0, 4.0, 5.0, 7.0 and 9.0 hours after the administration of single dose of Famotidine. Heparinized Blood Samples were centrifuged at 2500 rpm for 10 min to collect plasma. The plasma was then frozen at -

10°C until assay was performed. Samples were kept refrigerated during the period of analysis [8].

Sample Analysis

Mobile Phase Preparation

Chemicals Required

Methanol HPLC grade, Ortho Phosphoric Acid, Distilled Water

The mobile phase consisted of Methanol 200 ml/L, distilled water 800 ml/L and Ortho-Phosphoric Acid 0.5 ml/L. The mobile phase was sonicated and filtered by passing through filtration assembly under vacuum pressure using 0.45 µm membrane filter. The mobile phase was degassed by flushing with nitrogen through it for 2 minutes.

Method of Sample Analysis

Reagents Required

Famotidine Stock solution (100µg/ml) was prepared freshly on daily basis by dissolving appropriate amount of drug in Methanol.

Chromatographic Conditions

The analysis was performed by using Perkin Elmer High Performance Liquid Chromatography 200 series with UV-visible detector set at 220 nm. A reverse phase system was used consisting of a 5-µm Hypersil ODS (C18) column (250mm x 4.6 mm I.D.) preceded by 5-µm Hypersil ODS (C18) cartridge Guard (10mm x 4.6 mm I.D.) column. The mobile phase consisted of Methanol 20%, Distilled Water 80% and Ortho-Phosphoric Acid 0.05%, (Methanol : Water : Ortho-Phosphoric Acid;20:80:0.05) was filtered through 0.45 µm membrane filter, degassed by nitrogen flushing, and delivered at a rate of 1.0 ml/min. Injection of 20µl samples were injected, run time for famotidine was kept as 15 min [9].

Preparation of Standard Curve

Standard curve or calibration curve was made on daily basis. Blank plasma was spiked with famotidine drug solution to give the concentrations of 93.75, 187.5, 375, 750 and 1500 ng/ml. After deproteinization with Acetonitrile, 20 µl of sample was injected each time and spectra was taken for each concentration. The peak areas were noted for each concentration.

Preparation of Plasma Samples for Analysis

In 250µl of blood plasma sample, 250µl of Acetonitrile was added. Centrifuged at 5000 rpm for 10 minutes. After completion of deproteinization, plasma samples were filtered through Millipore filtration assembly. Collected the filtrate and injected 20µl of it directly into the HPLC. Obtained the spectra of blank plasma. Repeated this procedure for each blood plasma sample. From peak areas, calculated the concentration of drug with the help of statistical calculations.

Pharmacokinetic Parameters

Pharmacokinetic (PK) analysis was performed by using MS Excel XP with the help of Windows XP.

Maximum concentration of famotidine in plasma ($C_{\max}$) and time to these concentrations ($T_{\max}$) were determined from plasma concentration time profile. The area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) was calculated. All other pharmacokinetic parameters were also calculated.

Statistical Analysis

Statistical analysis was performed by using statistical software SPSS 12.

RESULTS

Standard Curve

Standard curves were constructed on daily basis. It is evident from the values in table that a clear and direct relationship between concentration and peak area exists. As the concentration increases, the peak area also increases in an almost linear manner. This trend indicates that the method is reliable and the results are consistent. Standard curve values are given in Table 1 and Figure 1.

Table 1: Standard curve values with peak areas.

Concentration	Peak Area
93.75	24450
187.5	48901
375	97804
750	195610
1500	391203

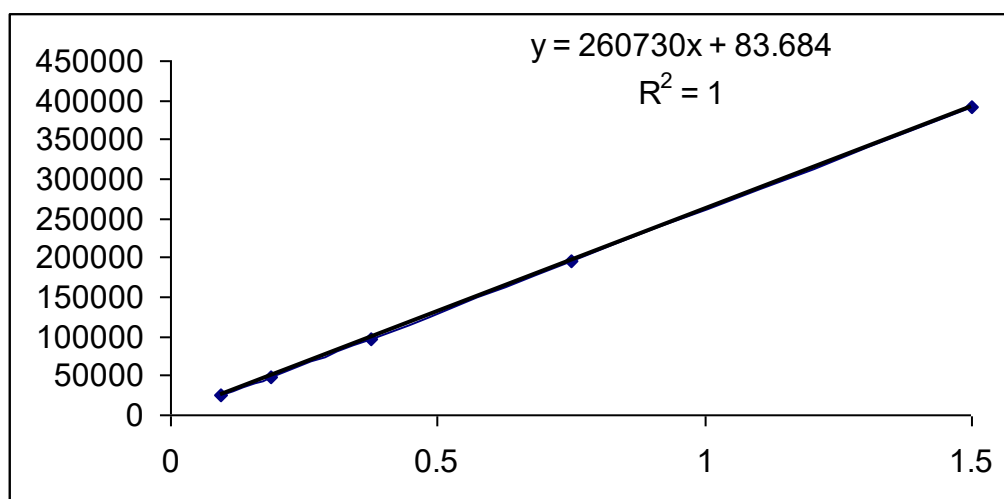


Figure 1: Graph between drug plasma concentration vs peak area.

Table 2: Pepsidine plasma concentration in twelve volunteers. (V = Volunteer)

Time (hrs)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0.50	0.936	1.060	1.200	1.086	0.865	0.794	1.000	0.800	0.860	0.990	0.880	0.880
1.00	1.220	1.220	1.350	1.286	1.350	1.200	1.380	1.140	1.250	1.320	1.430	1.540
1.50	1.450	1.600	1.790	1.579	1.600	1.840	1.540	1.650	1.580	1.650	1.780	1.780
2.00	1.088	1.088	1.950	1.746	1.500	1.800	1.800	1.670	1.540	1.500	1.600	1.430
3.00	0.923	0.923	1.700	1.320	1.460	1.029	1.270	1.400	1.250	1.300	1.400	1.200
4.00	0.681	0.681	1.480	0.990	1.306	0.842	1.080	1.340	1.230	1.300	1.120	1.100
5.00	0.553	0.553	1.350	0.730	0.866	0.753	0.880	1.100	1.050	1.190	0.990	1.030
7.00	0.382	0.382	1.220	0.640	0.587	0.660	0.620	0.500	0.680	0.840	0.640	0.800
9.00	0.234	0.170	0.530	0.320	0.347	0.271	0.530	0.130	0.370	0.440	0.410	0.360

Plasma Concentration in all Twelve Volunteers

The above table shows a clear trend of concentration/response over time in all volunteers: Initial increase was observed at 0.5 hour. Values were low at 0.50 hours (approximately 0.79–1.20), increasing to 1.14–1.54 at 1.00 hours. After initial increase a peak is observed. Highest values were observed between 1.50 and 2.00 hours in most volunteers, e.g. 1.95 in volunteer 3 (2.00 hours), 1.84 in volunteer 6 (1.50 hours) and around 1.6–1.8 in several others. Then as expected a gradual decrease can be seen. Values start to decrease significantly after 3.00 hours (approximately 0.92–1.70). a significant Decline was noted. Significant declines were observed in all volunteers from 5:00 to 9:00 hours, with values decreasing to a range of 0.13–0.53 at 9:00 hours. The data show a clear Rise–Peak–Decline pattern, with a maximum effect at 1.5–2 hours and a continuous decline thereafter (Table 2). Plasma concentration and log concentrations are represented in figure 2 & 3.

Average Plasma Concentration ($\mu\text{g/ml}$) vs Time (hrs) Values

Table 3 and figures 4 & 5 shows a clear trend in the mean plasma concentration over time. The concentration at 0.50 hours is $0.946 \mu\text{g/ml}$ which increases to $1.307 \mu\text{g/ml}$ at 1.00 hours, while the maximum level of $1.653 \mu\text{g/ml}$ is achieved at 1.50 hours. This is followed by a slight decrease at 2.00 hours with a value of $1.559 \mu\text{g/ml}$. A steady decrease in concentration begins from 3.00 hours, where it decreases from $1.265 \mu\text{g/ml}$ to $1.096 \mu\text{g/ml}$ at 4.00 hours and $0.920 \mu\text{g/ml}$ at 5.00 hours. This decrease becomes more pronounced over a longer period and reaches $0.663 \mu\text{g/ml}$ at 7.00 hours while the lowest value of $0.427 \mu\text{g/ml}$ is observed at 9.00 hours. Overall, the data show a clear initial increase, maximum concentration in the middle time, and a gradual decrease trend thereafter.

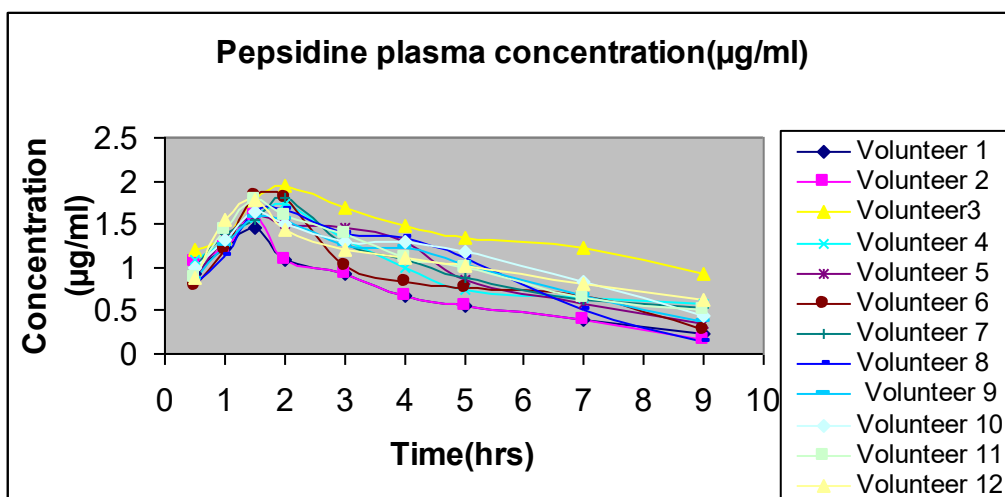


Figure 2: Cumulative plasma concentration of pepsidine® in twelve volunteers.

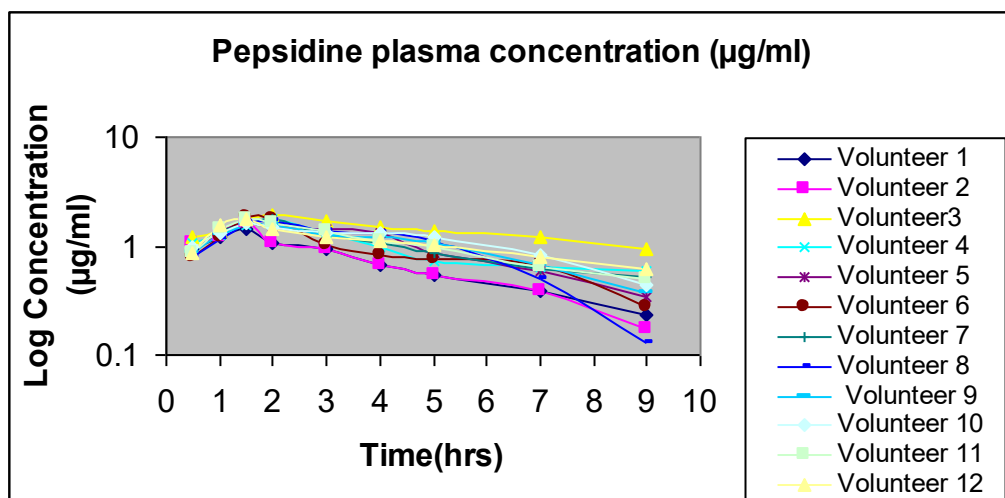


Figure 3: Cumulative log plasma concentration of pepsidine® in twelve volunteers.

Table 3: Pepsidine® average plasma concentration (µg/ml) vs time (hrs) values.

Time(hrs)	Average plasma Concentration (µg/ml)
0.50	0.946
1.00	1.307
1.50	1.653
2.00	1.559
3.00	1.265
4.00	1.096
5.00	0.920
7.00	0.663
9.00	0.427

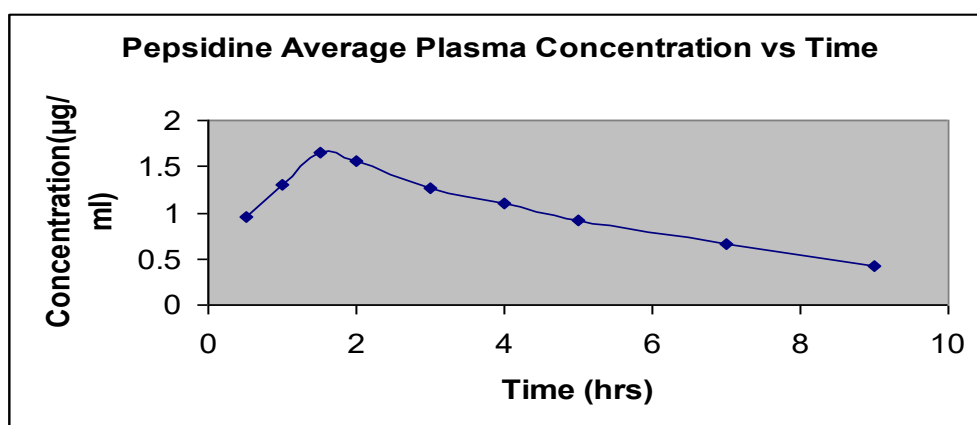


Figure 4: Graph between pepsidine® average plasma values and time (hrs).

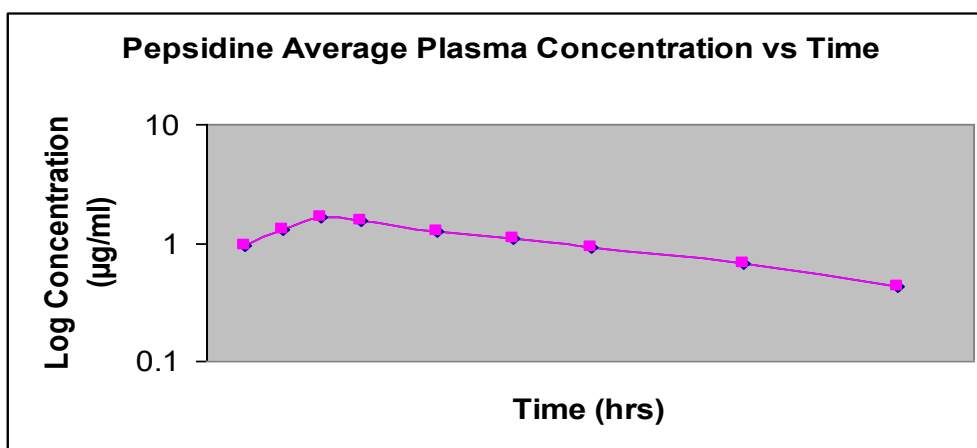


Figure 5: Graph between log pepsidine® average plasma values and time (hrs).

Pharmacokinetic Bio-parameters of Pepsidine

The overall trend of the main pharmacokinetic parameters across all volunteers is evident in table 4. The mean AUC (0–∞) was 11.48 and AUMC (0–∞) was 43.87, indicating overall drug exposure and retention. The mean value of maximum plasma concentration C_{max} was 1.559 µg/ml, while T_{max} was between 1.5 and 2.0 hours in most volunteers, indicating rapid absorption. The mean MRT was recorded as 3.75 hours, indicating that the drug was present in the body for a moderate period. The

elimination constant K_e averaged 0.394 hr⁻¹ and the elimination half-life t_{1/2} was approximately 1.98 hours, indicating relatively rapid elimination. The volume of distribution V_d averaged 13.2 and VSS approximately 1.0 L/kg, indicating adequate tissue distribution of the drug. Similarly, the mean Clearance (CL) was 3.60 ml/h/kg, showing a relatively consistent trend across volunteers. Overall, the data confirms a trend of uniform absorption, adequate distribution, and balanced excretion with low variability (SD and SEM).

Table 4: Pharmacokinetic bio-parameters of pepsidine®.

Volunteer	AUMC (0-∞)	AUC (0-∞)	C _{max}	T _{max}	MRT	K _e (Hr ⁻¹)	t _{1/2} (el)	V _d	VSS (L/Kg)	CL (ml/h/kg)
1	29.674	8.55	1.088	1.500	3.469	0.288	2.404	16.225	1.348	4.677
2	24.027	8.04	1.088	1.500	2.988	0.335	2.071	14.863	1.665	4.974
3	64.49	15.38	1.95	2.000	4.193	0.239	2.906	10.904	0.620	2.601
4	41.889	11.17	1.746	2.000	3.748	0.267	2.597	13.413	0.955	3.579
5	43.552	11.52	1.5	1.500	3.779	0.265	2.619	13.113	0.918	3.470
6	35.892	10.32	1.8	1.500	3.476	0.288	2.409	13.462	1.114	3.873
7	70.166	14.28	1.8	2.000	4.911	0.204	3.403	13.749	0.570	2.800
8	28.591	10.21	1.67	2.000	2.798	0.357	1.939	10.955	1.399	3.915
9	44.194	11.59	1.54	1.500	3.811	0.262	2.641	13.148	0.905	3.450
10	51.412	12.73	1.5	1.500	4.036	0.248	2.797	12.675	0.778	3.140
11	48.325	12.18	1.6	1.500	3.965	0.252	2.748	13.016	0.828	3.282
12	44.218	11.73	1.43	1.500	3.768	0.265	2.612	12.847	0.905	3.409
SUM	526.43	137.76	18.712	20.000	44.943	3.269	31.146	158.37	12.005	43.17
MEAN	43.869	11.48	1.559	1.667	3.745	0.394	1.979	13.198	1.000	3.5975
±S.D.	13.784	2.10	0.267	0.246	0.549	0.041	0.381	1.449	0.326	0.691
±SEM	3.979	0.607	0.077	0.071	0.159	0.012	0.110	0.418	0.094	0.199

DISCUSSION

In children plasma clearance $Cl_{(ml/h/kg)}$ and renal clearance (Cl_R) were found to be 0.70 ± 0.34 L/hr/kg and 0.43 ± 0.24 L/hr/kg, respectively. The elimination half-life $T_{1/2}$ was 3.2 ± 3.0 hours in children. In another study conducted on normal subjects apparent volume of distribution at steady-state (V_{ss}) was 2.4 ± 1.7 L/kg. In a study by James et al, in 1998 conducted on children maximum plasma concentration (C_{max}) was found to be 640.66 ± 250.66 ng/mL, elimination half-life $T_{1/2}$ was 10.51 ± 5.43 hours and apparent volume of distribution at steady state (V_{ss}) was 0.82 ± 0.29 L/kg.

The reported mean ($\pm$ SD) maximum serum concentration (C_{max}) for adults was 527.6 ± 281.2 ng/mL. In normal volunteers' maximum plasma concentrations of 104 ± 39 ng/ml has already been reported [10]. In another study conducted on the male human subjects it was reported 744 ± 372 ng.h/ml (10). In pervious studies conducted on normal subjects' clearance values ranged from 0.3-0.48 L/hr/kg. In a pervious study on normal subjects' elimination half-life was calculated with average values ranging from 2.2 to 3.3 hours [11]. In present study these values were found to be higher. These might be due to the inclusion of young human volunteers in the study. As age has a great impact on the plasma levels of the drugs. Hence it is possible that these high plasma levels would be achieved due to age factor. In previous study conducted it was pointed out that age could be one of the prominent factors which could increase the plasma levels of the drugs. Secondly, body surface area would have a great impact on the

drug absorption and hence raise plasma levels of the drug. All the human volunteers in the present study were of average normal body surface area. Hence their absorption of drug was rapid and had higher values. The effect of body surface area on drug absorption has been documented and pointed out that body upper extremes of body size were associated with increased clearance for both drugs. The pharmacokinetics and bioavailability of famotidine were investigated in healthy Chinese volunteers [12]. Linear pharmacokinetics were observed following either IV or PO administration. After 20 mg IV, plasma levels declined exponentially with an initial $T_{1/2}$ of 0.3 ± 0.2 h and terminal $T_{1/2}$ of 3.5 ± 0.8 h. The plasma elimination $T_{1/2}$ following PO administration were 3.4 ± 0.7 h and 3.5 ± 0.5 h for capsules and tablets respectively [13]. A study was conducted to describe the relationship between pharmacokinetics and body surface area (BSA). It was found that the area under the concentration-time curve (AUC) was significantly correlated with sex and age.

In a study on patients T_{max} were reported to be 3.6 ± 1.9 hours while in control it was 2.3 ± 1.05 hours (Maish et al., 1998). In patients of cystic fibrosis, the total clearance $Cl_{(ml/h/kg)}$ was 0.79 ± 0.41 L/kg/hr, the renal clearance was 0.57 ± 0.26 L/hr/kg (14). In a study by Maish et al., 1998 apparent volume of distribution (VD) in male patients was 1.33 ± 0.53 L/kg. In our results higher values of volume of distribution could be the result of average body surface area as well as lower BMI. Healthy human volunteers included in our study are:

In a study on the pharmacokinetics of famotidine in patients with cirrhosis and ascitis AUC of patients was found to be 837 ± 300 ng h/ml and AUC in control subjects was found to be 630 ± 204 ng h/ml [14]. Famotidine has an elimination half-life of 2.5-3.5 hours in normal subjects [15].

In present study these higher plasma values might be due to the factors as described earlier. In present study the higher absorption might have contributed to the higher mean residence time of the Famotidine.

Our volunteers were given the grapefruit. It is possible that famotidine AUC higher values are also due to the drug food interaction as it is mentioned in old literature. The factors which affected AUC are also interplaying their role in the higher AUMC values. 1. Young and aged between 18-33 years, and 2. They have average body surface areas. Hence it is possible that they might have higher volume of distribution values.

As far as Volume of Steady State (V_{ss}) is concerned, there is no difference between the values of literature and our findings. Regarding Half Life ($T_{1/2}$) In present study the plasma half-life of Pepsidine® was from 1.939 -3.403 hours with mean 1.979 ± 1.110 hours these values are consistent with the pervious studies conducted on famotidine in different situations. Elimination Rate Constant (K_e) In present study elimination rate constant i.e. K_e of the Pepsidine® was 0.204-0.357 with mean 0.394 ± 0.012 These high elimination rate constant values might be to the inclusion of young human volunteers in the study. All the human volunteers in the present study were of average normal body surface area. It is obvious that aforementioned

factors are responsible for the high elimination rate constant values of the famotidine in our healthy human volunteers. Clearance $Cl_{(ml/h/kg)}$ values for Pepsidine® were somewhat higher in our study than those values which were obtained in previous studies. It is obvious from above mentioned discussion that aforementioned factors are responsible for the higher plasma drug clearance values of the famotidine.

CONCLUSION

From aforesaid data, it is obvious that Famotidine 40 mg tablet in healthy human volunteers produced greater blood plasma values as compared to that given in the previous literature. These differences indicate that racial differences, geographic differences, nutritional differences, age, sex, gastrointestinal motility, physico-chemical properties of drug, polymorphism of drug, excipients, body surface area of the volunteers and most important the metabolic state of the human body is the main determinants of the pharmacokinetic and pharmacodynamic properties of the famotidine.

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Conflict of Interest: There is no conflict of interest.

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