5.0 DISCUSSION

Olopatadine hydrochloride is a new selective histamine H1 receptor antagonist with inhibitory effects on the release of tachykinins and inflammatory lipid mediators such as histamine, arachidonic acid, thromboxane and leukotriene (76).

Olopatadine is available in its conventional immediate release (IR) form to be administered twice a day. The advantages of extended release (ER) formulations over conventional dosage forms are improved patient compliance due to less frequent dosing, reduction in fluctuation in steady state levels therefore there is better control of disease condition and reduced intensity of local and systemic side effects (10, 80, 81). Extended release formulations provide higher maximum plasma concentrations with lower inter-patient variability than the conventional, immediate release (IR), twice-daily formulations. Additionally, therapeutic drug levels with ER formulations achieved rapidly and maintained over the course of 24 h, allowing once-daily dosing. The studies have also confirmed good tolerability and safety of ER formulations similar to the IR formulations (2). Under ideal conditions, an ER formulation maintains therapeutic blood level of a drug for a specific period of time. Oral controlled-release dosage forms have been developed and studied to restrict these systems to specific regions of the gastrointestinal tract as well as to improve the pharmacological activity and to reduce toxic effects (2, 3). ER formulation of a short-acting drug lead to better disease control than a corresponding immediate-release (IR) formulation at the equivalent total daily dosage (6, 7). Another undoubted advantage of ER formulation is improved patient compliance. Compliance improves dramatically as prescribed dose frequency decreases (8-11). It also increases the safety margins due to better control of plasma levels and causes maximum utilization of drug enabling reduction in total dose, thereby reducing
health care costs. The olopatadine extended release formulation is developed with the aim to achieve 1. Improved patient compliance by reducing dosing frequency, 2. Reduction in fluctuation in steady state level and therefore better control of allergic symptoms, 3. Reduction in intensity of local or systemic side effects. In many cases the ER drug delivery system increases bioavailability and thus increasing the area under the curve (AUC). However, in some cases, ER systems also reduces bioavailability but is compensated by other advantages like reduced fluctuations leading to better control over symptoms (82, 83).

The purpose of the present study therefore was:

- To determine the rate and extent of absorption of single dose of olopatadine hydrochloride 10 mg extended release tablet (two formulations) and two doses of Allelock® 5 mg immediate release tablets (each dose containing olopatadine hydrochloride 5 mg administered 12 hourly; total dose 10 mg), in healthy, adult, human male subjects under fed condition and

- To compare the pharmacokinetic parameters of extended release formulations with immediate release formulation in order to achieve the motive of reduced fluctuation in plasma level, which ultimately helps to combat disease symptoms more effectively and switching from twice daily dosing to once daily dosing so as to improve patient compliance.

The study was carried out in accordance with the basic principles defined in US 21CFR Part 320, USFDA guidance for industry for conducting bioavailability and bioequivalence Studies for orally administered drug products--general consideration 2003, the ICH (62 FR25692, 09 May 1997)’ Guidance for ‘Good Clinical Practice’, ICMR ‘ethical guidelines for biomedical research on human participants (2006)’, CDSCO ‘guidance for Good Clinical Practices for
Clinical Research in India’ and the principles enunciated in the Declaration of Helsinki (WMA General Assembly, Seoul, 2008) (17, 19, 22, 77, 78, 79). The study protocol was approved by the Jamia Hamdard Institutional Review Board.

The study was designed as an open label, balanced, randomized, three-treatment, three-period, three-sequence, crossover comparative bioavailability study under fed condition. Crossover design was utilized as in crossover design, clearance, volume of distribution, and absorption, as determined by physiological variables (e.g. gastric emptying, motility, pH), are assumed to have less inter-occasion variability compared to the variability arising from formulation performance. Therefore, differences between two products because of formulation factors can be determined. As it was a comparative bioavailability (BA) study in healthy subjects with pharmacokinetic end-points, blinding was not required so an open-label design was selected. Because single-dose studies are considered more sensitive in addressing the primary question of bio-equivalence (BE) (i.e., release of the drug substance from the drug product into the systemic circulation) therefore a single dose study was conducted. Co-administration of food with oral drug products may influence drug BA and/or BE and regulatory agencies recommend that fed study should be conducted for extended release formulations, so study was conducted under fed conditions (17).

Subjects were explained through oral presentation about the study and each of the subjects read and understood the information before giving his consent to participate in the study by signing the informed consent form. The signed original copy was retained and one signed copy was given to the study subject for the record. Subjects were screened and fifteen subjects were enrolled into the study as per the inclusion/exclusion criteria given in protocol. The mean age, weight and height of the subjects were 26.07 ± 6.62 years (ranged from 18 -
38 years), 57.17 ± 6.68 Kg (ranged from 46.9 - 69.5 kg) and 167.39 ± 7.63 cm (ranged from 156 – 185 cm) respectively. The subjects were adequately compensated on account of their participation in the study as per the guidelines issued by the Jamia Hamdard Institutional Review Board.

Treatment allocation was done on the basis of SAS generated randomization schedule, random allocation of the treatment was done to remove the bias in treatment assignment. Regulatory agencies recommend that a high calorie meal with high fat content should be utilized for fed studies so subjects were dosed approximately 45 minutes after the start of high fat high calorie meal.

It is recommended by regulatory authorities that blood samples be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug, for most drugs, 12 to 18 samples, including a pre-dose sample should be collected per subject per dose, this sampling can continue for at least three or more terminal half-lives of the drug and an adequate washout period (e.g., more than 5 half-lives of the moieties to be measured) should separate each treatment (17). On this basis the blood sampling schedule was designed and samples were collected till 36.0 hrs post-dose and a washout period of 6 days was given between each period on the basis of reported elimination half-life of olopatadine i.e. ~8 hrs. To avoid any degradation of the drug molecule by exposure to light and temperature, all the samples were collected and processed under low light condition and stored at below -50ºC.

Vital signs of oral temperature, sitting blood pressure and radial pulse were found to be normal for all the subjects during the course of the study in all periods of the study. The clinical examination of all subjects was found out to be normal. The study treatments were well tolerated by the study subjects. Only one subject (sub. no.12) experienced some adverse
event which was not serious in nature. Thirteen (13) subjects completed all three periods of the study except for subject no. 12 & 08 as they were withdrawn from the study due to adverse event and non-compliance to protocol respectively.

A liquid chromatography mass spectrometric (LC-MS/MS) method for the estimation of olopatadine in human plasma using olopatadine-d3 as an internal standard (ISTD) was developed and validated. The validation of this procedure was performed in order to evaluate the method in terms of selectivity, linearity, precision, accuracy, sensitivity, recovery and stability (48). The linearity, precision and accuracy evaluations were performed on three batches of spiked plasma samples.

The procedure involved solid phase extraction with oasis HLB 1CC cartridges. The drug and the ISTD were eluted from a zorbax eclipse XDB C18, 100x4.6mm, 3.5µM column at 30°C with a mobile phase consisting of 0.02% formic acid solution:methanol (40:60) (v/v) at a flow rate of 1 mL/min. mass spectrometric detector was used to measure the drug and ISTD using multiple reaction monitoring. Each analysis require no longer than 2.5 minutes. Quantification was achieved by measurement of the peak area ratio of the drug to the ISTD. The limit of the quantification of olopatadine in human plasma was 1.0023 ng/mL. The % bias values (compared to nominal) for intra-run accuracy of olopatadine LOQQC ranged from -12.17% to -4.95%, while the intra-run precision of LOQQC ranged from 3.92% to 13.00%. The % bias value for inter-run accuracy of LOQQC was -8.27%, while inter-run precision of LOQQC was 8.20%. The % bias values for intra-run accuracy of olopatadine at LQC, LMQC, MQC and HQC ranged from -2.42% to 4.96%, while the corresponding intra-run precision ranged from 1.06% to 5.31%. The % bias values for inter-run accuracy of olopatadine at LQC, LMQC, MQC and HQC ranged from -0.93% to 3.46%, while the
corresponding inter-run precision ranged from 2.21% to 4.09%. The analytical recovery of olopatadine was 95.58% and for ISTD it was 102.98%. From stability studies, olopatadine was found to be stable for 6 hrs 30 min. at room temperature and for 29 hrs 22 min. at 4°C. Olopatadine in plasma was found to be stable for 5 freeze-thaw cycles. These results were within the acceptance limit of ≤15% and ±15% for precision and accuracy as per guidelines and all other results of validation parameters were in acceptable range as per regulatory guidelines. So, the method was reliable, reproducible and accurate.

Pharmacokinetic parameters $AUC_{0-t}$, $AUC_{0-24}$, $AUC_{0-\infty}$, $\frac{AUC_{0-t}}{AUC_{0-\infty}}$, $C_{max}$, $T_{max}$, $K_{el}$, $t_{1/2}$ were calculated for olopatadine using WinNonlin-Node version 5.0.1. A statistical analysis was performed on pharmacokinetic data using the SAS system.

Pharmacokinetic data obtained in this study showed that that maximum concentration ($C_{max}$) in plasma after olopatadine administration attained by extended release (ER) test formulations A & B (110.33 ng/ml & 112.38 ng/mL respectively) was higher than the $C_{max}$ of IR reference formulation R i.e. 65.18 ng/ml. $T_{max}$ for both extended release formulations A & B were 3.73 & 3.00 hrs respectively, whereas the $T_{max}$ for reference formulation was 8.42 hrs. There was statistically significant difference ($p<0.0001$) between the $C_{max}$ of test formulations A & B and reference formulations R and A/R ratio and B/R ratio was 151.09 & 167.96 which was much higher than the normal range.

Two peaks were observed in the mean plasma concentration and time curve of IR formulation R, this was due to the 12 hourly administration of the formulation.

In relation to the reference formulation the values obtained for test product A & B were lower with respect to area under the curve. As $AUC_{0-24}$ values for formulations A, B and R were 387.60, 377.32 & 403.64 ng.hr/ml, $AUC_{0-36}$ values were 385.56, 372.81 & 418.27
ng.hr/ml and AUC_{0-\infty} values were 393.96, 379.29 & 425.58 ng.hr/ml respectively. These results demonstrated that the ER formulations showed a similar extent of abortion as compared to the reference formulation and there were no statistically significant difference (p<0.0001) in AUC of test formulation A and B and reference R.

The A/R ratios for log transformed data for the pharmacokinetic parameters AUC_{0-36}, AUC_{0-24}, AUC_{0-\infty} were 91.08, 94.90, and 91.32 respectively. The elimination rate constant of product A (0.22 hours\(^{-1}\)) was found to be higher than reference product R (0.19 hours\(^{-1}\)). The 90% confidence intervals for log transformed data for C_{\text{max}}, AUC_{0-36}, AUC_{0-24} and AUC_{0-\infty} for the test product A vs. reference R (A/R) were 127.41-179.17, 86.37-96.05, 90.13-99.93 and 86.75-96.12 respectively. All the values were within the stated regulatory bioequivalence range of 80-125\% (17, 19) with the exception of C_{\text{max}}. So bioequivalence between test product A & reference product R cannot be established.

The B/R ratios for log transformed data for the pharmacokinetic parameters AUC_{0-36}, AUC_{0-24}, AUC_{0-\infty} were 89.63, 93.95, and 89.63 respectively. The elimination rate constant of product B (0.24 hours\(^{-1}\)) was found to be higher than reference product R (0.19 hours\(^{-1}\)). The 90% confidence intervals for log transformed data for C_{\text{max}}, AUC_{0-36}, AUC_{0-24} and AUC_{0-\infty} for the test product B vs. reference R (B/R) were 142.25-198.30, 85.11-95.39, 89.34-98.79 and 85.26-94.22 respectively. All the values were within the stated regulatory bioequivalence range of 80-125\% (17, 19) with the exception of C_{\text{max}}. So bioequivalence between test product B & reference product R cannot be established.

The intra-subject variability for the C_{\text{max}}, AUC_{0-36}, AUC_{0-24} and AUC_{0-\infty} was reported for log-transformed data. Overall, the intra-subject variability (expressed as % CV) for all the
products was less than 30%. Intra subject variability was 26.0, 8.0, 7.8 and 7.7 for $C_{\text{max}}$, $AUC_{0-36}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ respectively.

P values were reported for the pharmacokinetic parameters $C_{\text{max}}$, $AUC_{0-36}$, $AUC_{0-24}$ and $AUC_{0-\infty}$. ANOVA model indicated statistically significant treatment effect especially for $C_{\text{max}}$ which reported a p value of <0.0001. There was insignificant difference between $C_{\text{max}}$, $AUC_{0-36}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ for the period and sequence effects as the p value was found to be more than 0.05. High p value indicates that there was no period and sequence effect.

Power of the test for $C_{\text{max}}$, $AUC_{0-36}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ was found to be 59.15%, 100.00%, 100.00% and 100.00% respectively. The power of test was more than 80% for $AUC_{0-36}$, $AUC_{0-24}$ and $AUC_{0-\infty}$; however, it was less than 80% for $C_{\text{max}}$.

In this study, higher $C_{\text{max}}$ values of test formulations A & B were reported as compared to reference formulation R, according to statistical results, there was statistically significant difference in $C_{\text{max}}$ between reference formulation and test formulation A and B as A/R & B/R ratio values were 151.09 & 167.96 respectively which were higher than the defined regulatory range of 80% to 125%. In a comparative bioavailability study conducted to examine the pharmacokinetics of prochlorperazine immediate release tablet and sustained release tablet in healthy, adult, male volunteers, the reported $C_{\text{max}}$ values for sustained release tablet and immediate release tablet were 297.89 & 218.41 ng/mL respectively (84), the similar pattern is shown in our study where the $C_{\text{max}}$ values of the sustained release formulations were higher than the immediate release.

The reason for higher $C_{\text{max}}$ of test formulations A and B than the reference formulation was possibly due to the fact that in first 2-3 hours these formulations behaved similar to IR formulation and additionally because of higher dose than IR formulation; whereas after 3
hours of administration the test formulation A illustrated extended release pattern. Another possible reason of the higher $C_{\text{max}}$ values of extended release test formulations could be dose dumping. Dose dumping is defined as unintended, rapid drug release in a short period of time of the entire amount or a significant fraction of the drug contained in a modified release dosage from. Dose dumping can pose a significant risk to patients, either due to safety issue or diminished efficacy or both. Generally dose dumping is observed due to a compromise of the release-rate-controlling mechanism (85). Dose dumping is often reported when a modified oral dosage from is conjunction with high fat food or alcohol. Hendeles et al reported dose dumping phenomenon in a study conducted on theophylline extended release tablets taken under fed conditions. They reported that absorption of slow release theophylline after an overnight fast was very slow, with only 71% of the dose ultimately absorbed. In contrast, food caused precipitous dose-dumping resulting in dose normalized peak levels in in the serum that averaged 2.3 times higher than after a fasting dose. About half of the dose was absorbed in a four-hour period (86). Another study of nifedipine ER tablets in healthy volunteers reported an increase in plasma concentration of the test drug after a high fat breakfast. The test product developed a dose-dumping effect after the intake of food. Besides a resultant higher extent of bioavailability, reflected by an increase in the mean geometric AUC values, a significant increase in the geometric mean $C_{\text{max}}$ value was noted. This phenomenon went along with a loss in modified release characteristics. Concomitant food intake impairs the ability of the test formulation to release nifedipine in a regulated manner (87). US FDA also recommends when studying modified-release dosage form, consideration should be given to the possibility that co-administration food can result in dose dumping, in
which the complete dose may be more rapidly released from the dosage form than intended, creating a potential safety risk for the study subjects (17).

In our study, extended release formulations showed a similar pattern which indicates a possible dose dumping effect. Major portion of olopatadine from both the extended release test formulations was released in first few hours leading to a failure in the modified release characteristics of the formulation. The test formulations were not able to release the olopatadine in a regulated and controlled manner as expected from a modified/controlled release tablet. However, these higher C\text{max} values of extended release formulations cannot be confidently attributed to the dose dumping phenomenon, as to confirm this we need to perform a similarly designed study in fasted state with a similar set of subjects.

Another noteworthy observation in the study was that the maximum C\text{max} values for both the test formulations were reported from the same subject who is a regular smoker, so it is possible that regular smoking may have produced some changes in the normal physiological processes of the subject which ultimately caused the higher release of the of the drug from the ER formulations. Numerous drug interactions have been identified with tobacco smoke (88) and many regulatory agencies like Canadian, European and WHO recommend that preferably a non-smoker should be included in the bioequivalence studies (18, 30, 41). But this can only be confirmed when another study in planned with both smoker and non-smoker subjects to study the effect of smoking on the pharmacokinetic parameters of olopatadine.

Regulatory agencies typically require that bioequivalence studies should be sufficiently powered i.e. at-least 80%. This means that there is 20% probability of not demonstrating bioequivalence even if the two formulations are truly bioequivalent. The number of subjects required ensuring a power of 80% with an α error of 5% is based on the variability of the
metrics that the study must pass on. It is well known that most variable metric in bioequivalence studies is usually the $C_{\text{max}}$ and studies most often fail because of $C_{\text{max}}$ as the intra-subject variability increases more number of subjects are required to meet the bioequivalence criteria. E.g. With 10% intra-subject CV for $C_{\text{max}}$, only 8 subjects will be required in a crossover design to prove bioequivalence with 80% power but with 25% intra-subject CV, 28 subjects will be required (89). In this study the power for $C_{\text{max}}$ was 59.15% which is very less than 80% while other parameters attained 100% power. The reported less power for the $C_{\text{max}}$ can be attributed to the intra-subject variability. The intra-subject CV for $C_{\text{max}}$ was 26.0% which is on the moderately higher side as drugs with more than 30% CV are termed as highly variable drugs. As intra-subject CV increases, large numbers of subjects are required to attain a power of 80%. However, in this study the Test/Reference ratio for both the test formulations was very high (i.e. 151% for A and 167% for B), therefore it is quite unlikely that increase in the number of subjects will change the outcome of the study.

Out of the three variables, i.e. treatment, period and sequence, the treatment effect was clearly visible for all the parameters but it was very significant in terms of $C_{\text{max}}$. This shows that test formulations behaved differently as compared to reference formulation but as $C_{\text{max}}$ is the most variable parameter the effect was more prominent.

Other possible constraint responsible for these results may be the selection of small number of subjects as it was a pilot study. Nonetheless, these higher $C_{\text{max}}$ values of test formulation didn’t pose any safety issue, as only one subject in test arm reported post-dose gastrointestinal adverse events which were not serious in nature and subject recovered without sequelae.
So, on the basis of pharmacokinetic and clinical results, it can be summarized that extended release formulation A & B achieved similar AUC as compared to reference drug but in both the test formulations most of the drug got released in initial few hours resulting in higher $C_{\text{max}}$ values but there were no safety concerns. Although the extended release formulation have showed a similar extent of absorption but the products need to reformulated in such a manner that slow drug release can be achieved as shown by lower $C_{\text{max}}$ and longer $T_{\text{max}}$. 