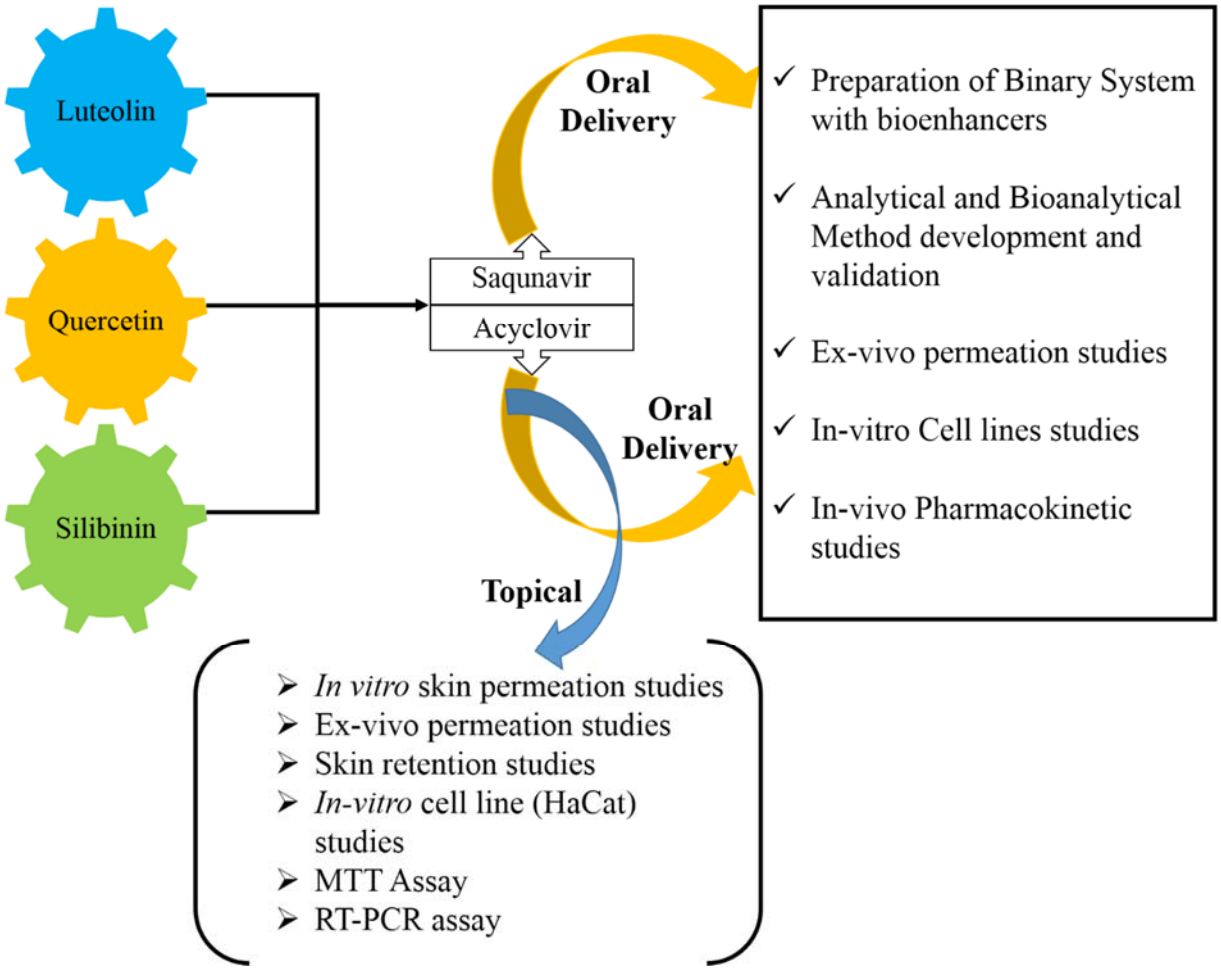
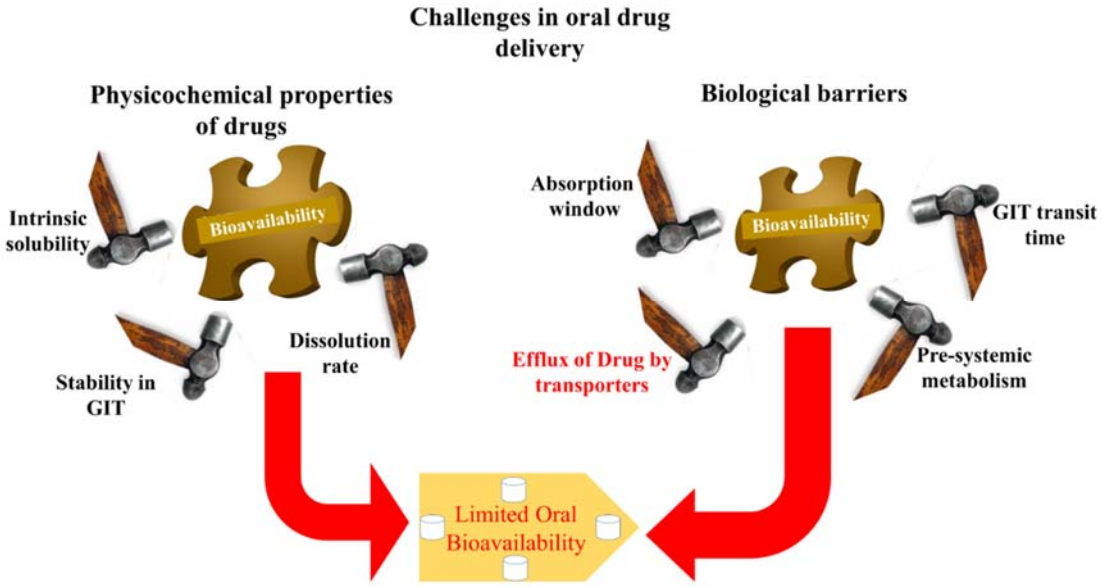
Graphical presentation of Chapter VII

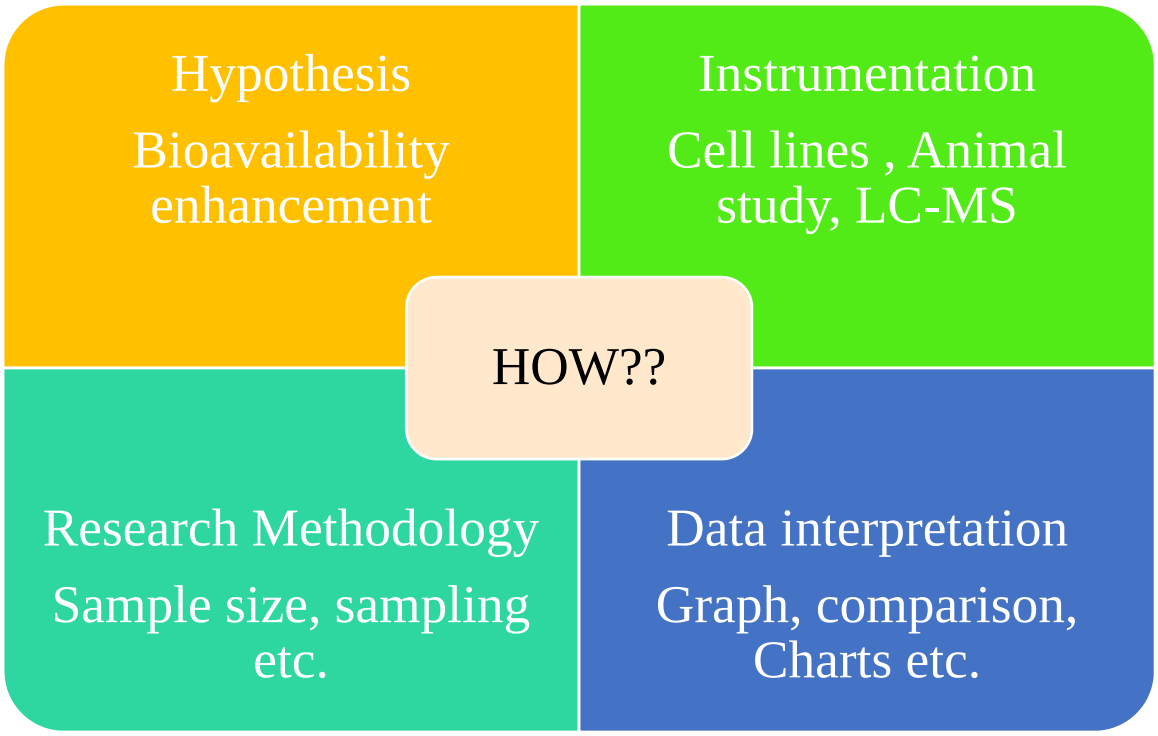


In **Chapter-I**, a detailed introduction has been provided about the state of the art of the problem, past experimental work, issues with the bioavailability, role of different factors on bioavailability, several techniques to overcome the issues of the bioavailability and all other important literatures has been described in the chapter to have thorough knowledge of the research topic. The literature review has been done thoroughly with a care of every aspect of the research topic such as techniques to be used in experimental section etc.



In **Chapter-II**, as from chapter-I we got a strong building blocks for research work, in this chapter the work has been planned and categorize wise. During the designing of this part all the techniques has been thoroughly studied and compared and the best ones were chosen for the experimental section. In this chapter, hypothesis was studied using the acyclovir (ACV) and saquinavir (SQU) has been chosen as the anti-viral drugs as these both drugs are potent and most important drugs in their respective category of herpes simplex virus and protease inhibition respectively and Quercetin (QU), Silibnin (Sil) and Luteolin (LT). In this part specific aim of the research work has been discussed which is to study the effect

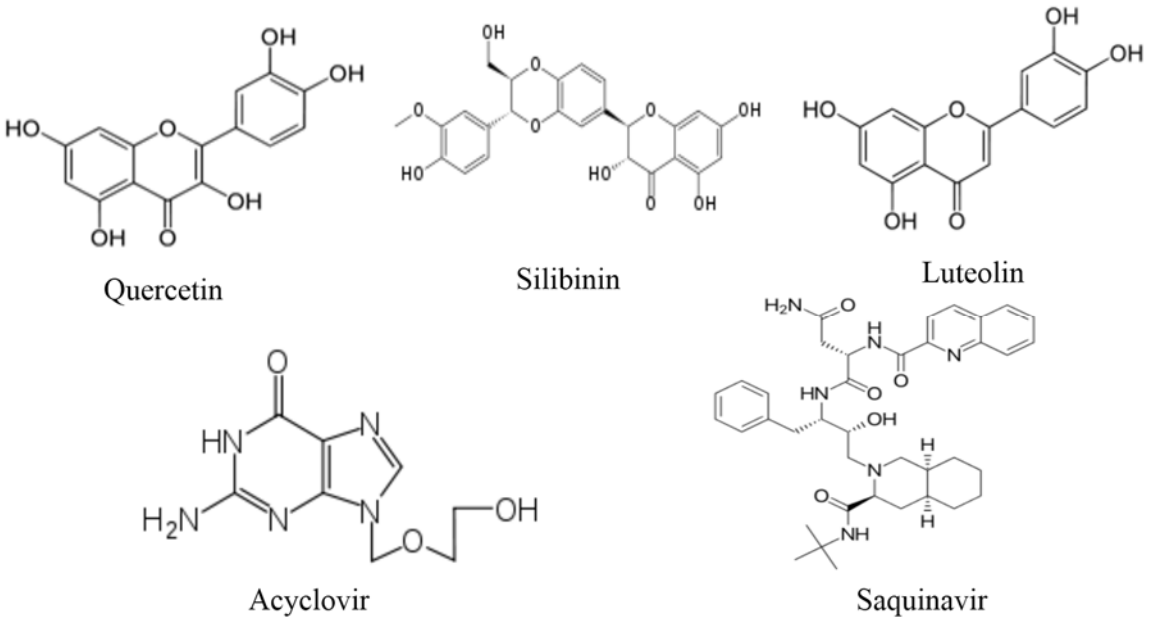
of natural bioenhancers on the bioavailability of acyclovir and saquinavir. The experimental parts includes analytical profiling, permeation studies, pharmacokinetic studies.



In **Chapter-III**, as before starting the research work it is prime focus of the researcher to have maximum information about the drug candidate's he is working on. In this chapter, a dig on the information about drugs and bioenhancers has been done, and all the important and relevant aspects for the study has been incorporated in the chapter such as structure, bioavailability, dose mechanism of action and all other details has been provided in this chapter.

Acyclovir (ACV) is one of the utmost used antiviral agents. It is an acyclic guanosine derivative. It has been used in the treatment of genital herpes, herpes simplex and neonatal HSV infection. It has been shown that acyclovir has high solubility and low intestinal

permeability and considered as a typical class III drug according to Bio-pharmaceutics Classification System (BCS) from the Food and Drug Administration (FDA) of the United States. The oral bioavailability is approx 20% with a Half–life of Plasma, 2 to 3 h. Volume of distribution is 4L to 55 L/1.73 m2 With Protein binding in plasma is 9 to 33%. It is administred in the dose of Up to 30 mg/kg body weight daily intravenously; up to 4 g daily by mouth.



Saquinavir (SQU) was the first drug candidate which gets approved as protease inhibitior for the HIV infection treatment. In the last decade, SQU has become a chief component of highly active antiretroviral therapies. SQU having molecular weight 670.8 with partition coefficient (LogP) 4.1. It is usually administered in the salt form mesylate, the solubility of the SQU is pH-dependent. Apart from having the not very much favorable physicochemical properties for permeability, it is also reported that efflux protein (P-gp) also hampers the transport of SQU from the gut wall. Inspite of this SQU is also

metabolized by both human hepatic and small intestinal enzymes, which also further results in low oral bioavailability (4%) and displays wide inter-individual variability.

Quercetin (QU) is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It exhibited activities including antioxidant, radical scavenging, anti-inflammatory, antiathero sclerotic, anticancer, and antiviral effects. It is a potent inhibitor of CYP3A4 and a modulator of P-glycoprotein.

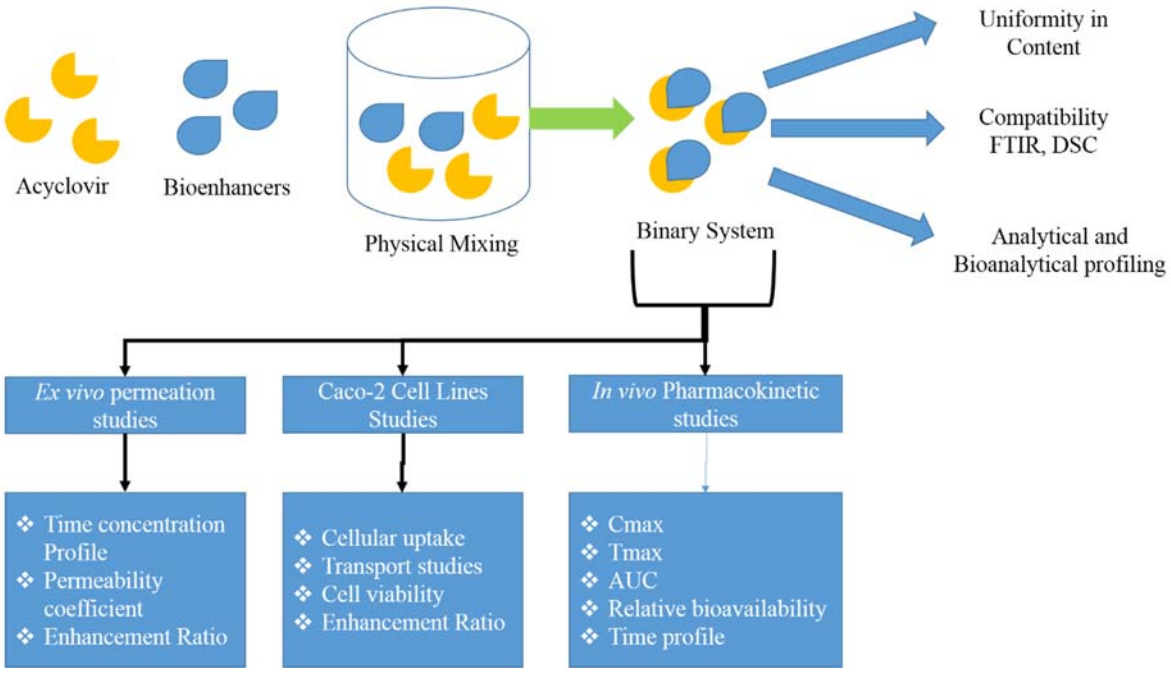
Silibinin (Sil) is the one of the chief and mostly abundant component approximately 70% of silymarin. It has been reported that silibinin could inhibit human CYP1A2, CYP2D6 and 3A4 enzymes responsible for the metabolism. Silibinin has also been reported as P-gp inhibitor.

Luteolin (LT) is one of the main constituents of P. barbatus herbal tea. However, there is a very little known about the way that these compounds affect the bioavailability.

Nevertheless, it is known that flavonoids interact with transport systems in intestinal cells, such as the ABC transporters P-glycoprotein (Pgp) and multidrug resistance proteins (MRP), which actively inhibit them. The major reason to choice these bioenhancers is their P-gp inhibition activity and to explore new ways to make more effective oral therapies for HIV infection as the major issue with the SQU is the P-gp efflux pump.

Chapter-IV, is about the oral uptake enhancement of the ACV, in this chapter the detailed studied carried out. ACV binary systems were prepared using different weight ratios of three bioenhancers 0.5-3%. Binary systems were prepared by means of physical mixing method. The prepared binary mixtures were evaluated for uniform of content using analytical method and compatibility using FTIR and DSC. The results of uniformity of content shows result in the range of 99-101% which shows the mixture contains uniform

amount of ACV. In the FTIR the characteristics peak of ACV were compared and there was no change observed in the peaks (intensities varies) which represents no interaction between the ACV and bioenhancers. These results were further confirm by DSC which shows a minimal change in the peak with respect to melting point. DSC results confirms there is no strong interaction between the ACV and compound. These findings encourages the research work.



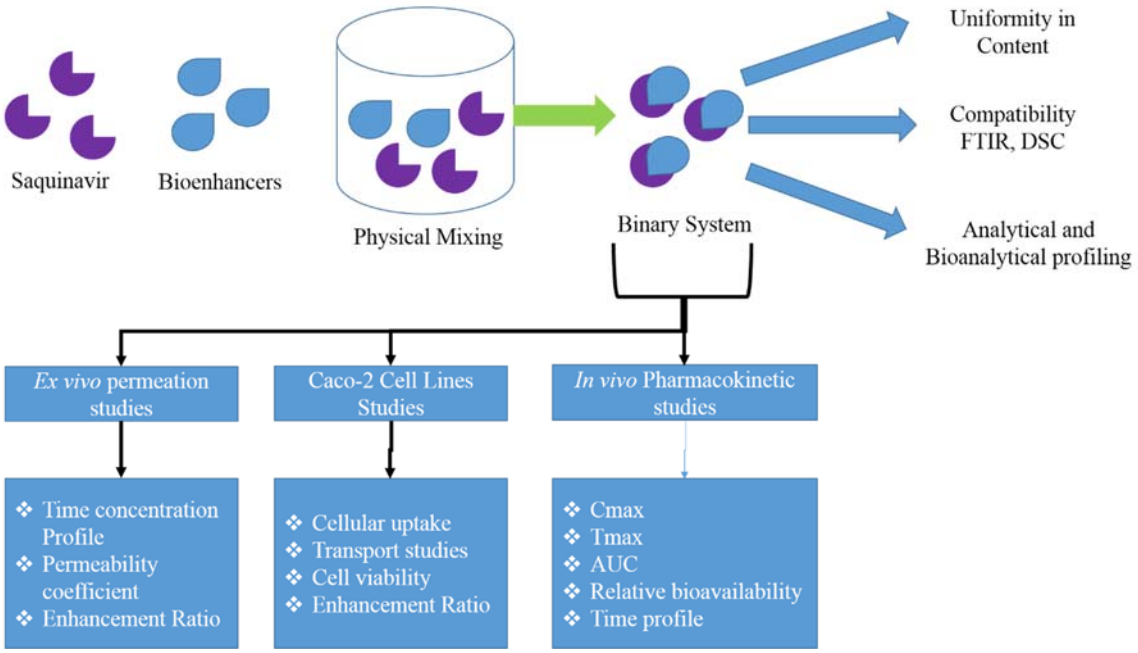
Time concentration profile of *ex-vivo* studies shows there is increase in the concentration of the ACV with the time and also the concentration was high in the binary system. The permeation coefficient calculated confirms the increase in the permeation in binary sytem. The permeation coefficient in plain ACV was $(0.435 \pm 0.02) \times 10^{-6}$ cm/s. QU shows an increase in the amount permeated with having permeation coefficient $(0.675 \pm 0.02) \times 10^{-6}$ cm/s at weight ratios (5:2), while Sil shows maximum permeation coefficient (0.682 ± 0.01) × 10-6 cm/s at weight ratios (5:1) and LT shows maximum enhancement at weight

ratio (5:2.5) with permeation coefficient (0.621 ± 0.03) × 10-6 cm/s. These results primary confirms the increase in the permeation.

Cellular uptake studies qualitatively using CLSM, confirms the uptake of the ACV in the Caco-2 cell lines. It also shows that the individual particles increase in the case of the binary system. Transport studies across the monolayer revealed that there is increase in the amount permeated through the layer with the time. The TEER was measure pre and post experiment. The variation in the TEER was not on the higher sides which further confirms there was no cellular level damage by the bioenhancers. The Papp (AP to BL side) of ACV and its binary systems were calculated and maximum Papp was observed with the QU in the ratio of 5:2 while in the Sil and LT maximum was observed in the ratio of 5:2 and 5:2.5 respectively. Although it was also observed that the ratio of Sil 5:1.5 was very close, but 5:2 concentration also shows maximum enhancement in the ex-vivo studies so this was chosen as optimum concentration for pharmacokinetic studies.

In-vivo pharmacokinetic studies were conducted on ACV and ACV:QU (5:2), ACV:Sil (5:1) and ACV:LT (5:2.5) binary systems. AUC increase by 4.87 folds in the case of the QU. While, 3.22 and 2.74 folds increase was observed with the Sil and LT respectively. The increase in the AUC confirms the utilization of the bioenhancers as the bioavailability enhancer for ACV. These bioenhancers can be given as food supplements or can be incorporated with the ACV for herpes simplex therapy. These studies clearly shows that the incorporation of such molecules can increase the effectiveness of herpes simplex virus treatment therapy.

In **Chapter V**, effect of bioenhancers on the SQU has been studied in detail. SQU binary systems were prepared using different weight ratios of three bioenhancers 0.5-3%. Binary systems were prepared by means of physical mixing method. The prepared binary mixtures were evaluated for uniform of content using analytical method and compatibility using FTIR and DSC. The results of uniformity of content shows result in the range of 98-100% which shows the mixture contains uniform amount of SQU. In the FTIR the characteristics peak of SQU were compared and there was no change observed in the peaks (intensities varies) which represents no interaction between the SQU and bioenhancers. These results were further confirm by DSC which shows a minimal change in the peak with respect to melting point. DSC results confirms there is no strong interaction between the SQU and compound.



Time concentration profile of *ex-vivo* studies shows there is increase in the concentration of the SQU with the time and also the concentration was high in the binary system. The

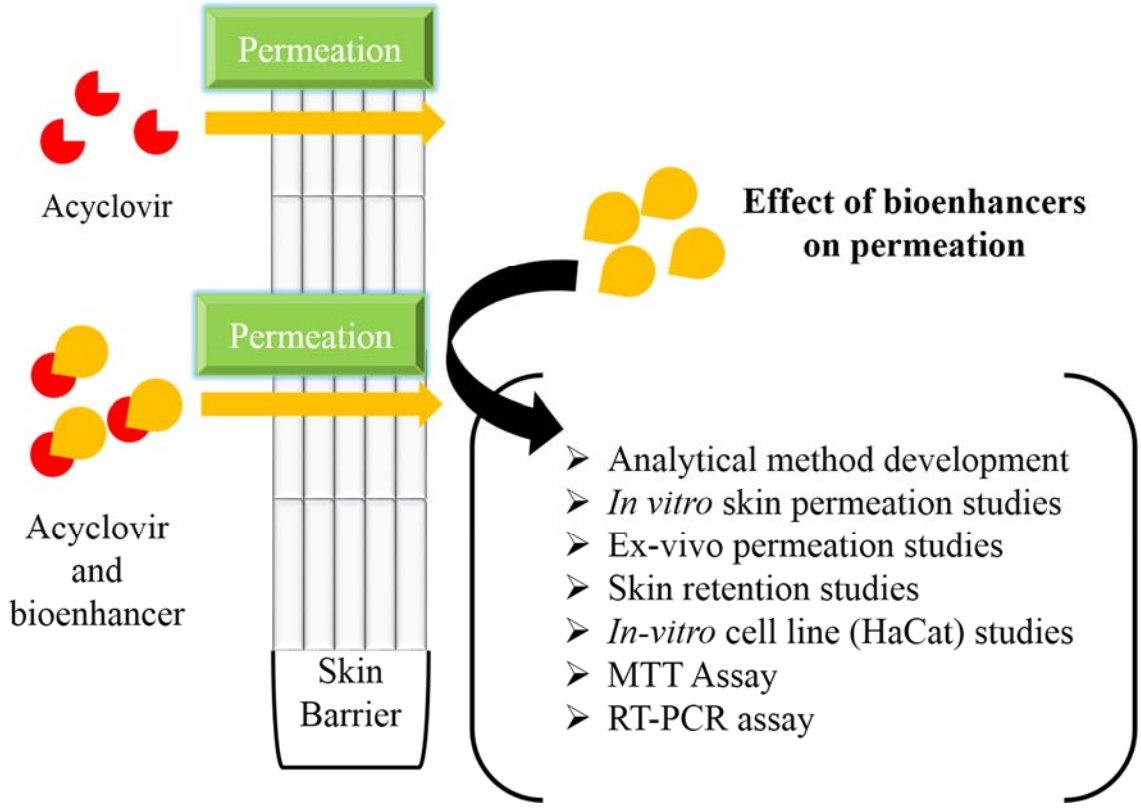
permeation coefficient calculated confirms the increase in the permeation in binary sytem. The permeation coefficient for plain SQU was (2.135±0.387) × 10^{-6} cm/s. The QU shows an increase in the amount permeated having permeation coefficient (4.395±0.15) × 10^{-6} cm/s at weight ratios (5:1), while Sil shows maximum permeation coefficient (4.283±0.18) × 10^{-6} cm/s at weight ratios (5:2) and LT shows maximum enhancement at weight ratio (5:2.5) with permeation coefficient (3.956±0.458) × 10^{-6} cm/s. These results primary confirms the increase in the permeation.

Cellular uptake studies qualitatively using CLSM, confirms the uptake of the SQU in the Caco-2 cell lines. It also shows that the individual particles increase in the case of the binary system. Transport studies across the monolayer revealed that there is increase in the amount permeated through the layer with the time. The TEER was measure pre and post experiment. The variation in the TEER was not on the higher sides which further confirms there was no cellular level damage by the bioenhancers. The *P*app (AP to BL side) of SQU and its binary systems were calculated for 24 hrs. As shown in (Figure 5.15, 5.16, 5.17) maximum *P*app was observed with the QU in the ratio of 5:1 while in the Sil and LT maximum was observed in the ration of 5:2 and 5:2.5 respectively. Although it was also observed that the ratio of Sil 5:2 and 5:2.5 was very close, but 5:2 concentration also shows maximum enhancement in the ex-vivo studies so this was chosen as optimum concentration for pharmacokinetic studies.

In-vivo pharmacokinetic studies were conducted on SQU and ACV:QU (5:1), ACV:Sil (5:2) and ACV:LT (5:2.5) binary systems. AUC increase by 2.51 folds in the case of the QU. While, 2.34 and 2.09 folds increase was observed with the Sil and LT respectively The increase in the AUC confirms the utilization of the bioenhancers as the bioavailability

enhancer for SQU. These bioenhancers can be given as food supplements or can be incorporated with the SQU for protease inhibition in anti-viral therapy. These studies clearly shows that the incorporation of such molecules can increase the effectiveness of anti-viral therapy.

Chapter VI, The bioenhancers QU, Sil and LT shows an increase in the permeation of ACV in the in-vitro permeation study, ex-vivo permeation and cell lines study.



The observations shows more increase in the flux of ACV at low concentration Sil (2%) and LT (1%) as compared to the high concentration (3, 4 and 5%). While in the QU more increment was observed at the higher concentrations (4%) as compare to lower. In HaCat cell line studies ACV:QU shows the maximum enhancement was found at 6 µM having 1.36 fold enhancement ratio. While in the ACV-Sil and ACV-LT maximum enhancement

was at 4 µM and 2 µM having 1.41 and 1.23 fold increase in the concentration of the drug respectively. MTT assay results shows the damage to the cell layer was minimum with the bioenhancers. The MTT assay observations also supports the previous observations as from MTT assay it has been observed that there is no toxicity due to QU, Sil and LT. The irritation studies revealed that no irritation caused by the bioenhancers on the skin, hence they are safer for the topical delivery. RT-PCR assay although not clearly represents the activity but a slight change in the expression of tight junctions revealed that there is need of cellular level study is further needed up to reveal the exact mechanism of the bioenhancers. In the comparison of the ER of the three bioenhancers it has been observed that the all of them shows almost same and makes all the bioenhancers can be used for permeation enhancement. Although Sil was on the higher edge as Sil is found to be more active as it shows activity in very less concentration as compare to the QU and LT. Sil can be concluded as the first choice of bioenhancers for topical permeation enhancement.

These promising observations from the all studies carried out excited the researchers to further explore the exact mechanism of the bioenhancers effect on the skin permeation as these molecules can dig up a new era for the topical delivery of the drugs with poor permeation and improve the therapy. The incorporation of these bioenhancers in the topical therapy of herpes simplex virus can improve the patient compliance and therapy for Herpes simplex virus. These studies open several paths for the researcher to expand and improve the topical therapy.