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Absorption enhancement effect of piperine and chitosan on ganciclovir solid lipid nanoparticles: formulation, optimization and invivo pharmacokinetics

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ABSTRACT

The purpose of the present study was to formulate Solid Lipid Nanoparticles (SLNs) of Ganciclovir (GCV) in combination with Chitosan and Piperine for absorption enhancement effect. GCV loaded SLNs were prepared by hot homogenization method, optimized and characterized. Formulated SLNs were incorporated with absorption enhancers and characterized for *invitro* absorption (with chicken intestine), histopathological and *invivo* pharmacokinetic studies. *Invitro* absorption studies revealed that the permeability coefficient of the prepared formulation is more when compared to the pure drug, so the permeability is more for prepared formulation. *In vivo* pharmacokinetic study showed a significant increase in the Cmax, AUC, biological half-life and decrease in elimination rate constant for prepared formulation compared to pure drug. Histopathological studies also showed mild reversible damage of epithelial cells with Chitosan which indicates the safety and efficacy of the formulation. Thus, GCV loaded SLNs prepared with Chitosan can be clinically promising for enhancing the oral, intestinal absorption of the said BCS Class-III drug.

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INTRODUCTION

The infection caused due to cytomegalovirus (CMV) is the major reason for morbidity and mortality in neonates and immunosuppressed patients. This virus is responsible for several diseases like retinitis, Pneumonitis and encephalitis. (Jungang Ren *et al*., 2013) Ganciclovir is the first antiviral drug to treat CMV infection in humans. (W.T. Ashton *et al*., 1982) There are no improved results due to GCV in case of encephalitis, and the viral concentrations in case of retinitis. (O.L. Laskin *et al*., 1987) So, the performance of the GCV appears to be inefficient due to its poor permeability through the intestinal barrier.

In very recent years, Solid Lipid Nanoparticles (SLNs) were believed to increase the intestinal absorption of poorly permeable drugs by lymphatic circulation. (D.B. Chen *et al*., 2001, R.C. Doijad *et al*., 2008) SLNs are a type of colloidal systems consisting of a solid lipid and a surfactant for stabilization of dispersion. The lipid must be a high melting point substance, and the surfactant must be aqueous. The particles diameter ranges from 50nm to 1000nm. (R.H. Muller *et al*., 2004) The reported literature for the preparation methods of SLNs speaks that the following methods like precipitation, (B. Heurtault *et al*., 2003) solvent evaporation, (W. Mehnert *et al*., 2001) hot homogenization (L. Hu *et al*., 2010) and microemulsion techniques.

(K.A. Shah *et al*., 2009) Among these methods, high-pressure hot homogenization was found to be easy and reproducible technique.

Chitosan is a cationic polysaccharide which shows the significant mucoadhesive property as well as drug penetration activity across various epithelia. (Giuseppina Sandri *et al*., 2017) Piperine is obtained from black and long pepper and gained mass attention among the researchers with its nature of increasing the bioavailability of poorly permeable drugs by different mechanisms like rupturing the tight junctions of epithelial cells and inhibition of efflux transporters. (Bi Xiaoli *et al*., 2019) So, Piperine and Chitosan are believed to be natural bioenhancers which are incorporated into the formulated SLNs for improving the permeability of GCV.

The goal of present research work is to develop SLNs of Ganciclovir with bioenhancers to improve the oral permeability thereby improved absorption and bioavailability. Furthermore, the prepared SLNs were subjected to *Invitro* absorption and *Invivo* pharmacokinetic studies to prove absorption enhancement.

MATERIALS AND METHODS

Ganciclovir is kindly gifted by Ranbaxy Research Laboratory Gurgaon, India. Glyceryl mono and distearate were purchased from Himedia Laboratories Pvt. Ltd. Mumbai. Compritol 888 and Poloxamer 188 was purchased from S.D. Fine Chemicals, Mumbai, which is used as the surfactant. Tween 80 was purchased from Yarrow Chemicals which was also used as the surfactant. All the chemical components used in the present work are of analytical grade.

Preparation of GCV loaded Solid Lipid Nanoparticles

Selection of lipid: Solubility study of different lipids in the drug was performed for lipid selection. Each lipid (Compritol 888, Glyceryl monostearate & Glyceryl distearate) was mixed separately with the active ingredient in two different ratios 1:2 & 1:3 in different test tubes. The mixture of lipid and drug were melted above the 5^0C melting point of lipid using a water bath. The test tubes were observed for miscibility. (Marzia Cirri *et al*., 2017)

Formulation of SLNs: Among the various methods available for the preparation of nanoparticles, High-pressure homogenization method has been used for years, and it is a powerful technique for the large scale production. So, this method is used in the preparation of SLNs in the present study. (Ibrahim A *et al.*, 2017)

In this, drug (GCV) and lipids like Compritol 888, Glyceryl monostearate and Glyceryl distearate were taken in a beaker, and on other hands, Poloxamer 188 with water is taken in another beaker. Both were heated at 75°C (above the melting point of lipid). Then the aqueous phase is added to the lipid phase gradually by shearing to obtain a primary emulsion. This was subjected to ultrasonication at 400 watts' power and 90% pulse for 15 minutes followed by subjecting it to High-pressure homogenization at 750 bars pressure for 3 cycles. The resultant dispersion is cooled at 18°C to produce SLNs. (Sri Vishnu Kiran Rompicherla *et al*., 2017)

Evaluation

Percent Entrapment Efficiency

The prepared SLNs were evaluated for %EE. To obtain this, about 10ml of SLN dispersion centrifuged in Remi cooling centrifuge which is rotated at 20,000 RPM for 2 hours. The obtained supernatant is analyzed at 261nm (n=3) by UV visible spectrophotometer. This will give the amount of drug present in the dispersion. The final %EE is calculated by the following mathematical expression. (Gokce Dicle Kalaycioglu *et al*., 2016).

$$\%EE = \frac{\text{Total amount of drug} - \text{amount of drug present in supernatant}}{\text{Total amount of drug}} \times 100$$

Particle size and Zeta potential

The particle size & Zeta potential gives information about the size of the particle in the dispersion and degree of aggregation of Nanoparticles respectively. This was analyzed (n=3) by an instrument called HORIBA zeta sizer. A little volume of SLN dispersion is diluted with purified water which is further filled in polystyrene cells and placed in particle size analyzer at a wavelength of 632nm. The scattering of light on the sample was observed at 173^0 angle at a temperature of 25^0C. The values of particle size and zeta potential were obtained from the software present in the instrument. (Giulia Graverini *et al*., 2018)

Transmission Electron Microscopy (TEM for morphological characterization)

The formulated Solid Lipid Nanoparticles were characterized for their morphological study by using Transmission Electron Microscopy. A drop of a mixture of SLN dispersion with phosphotungstic acid was kept on a copper grid which is previously coated with carbon. The sample was micrographed at 200kv on a digital TEM station. (Raj Kumar *et al*., 2018)

***In-vitro* release studies:** The *in-vitro* release of GCV SLN formulations is carried out by the dialysis bag diffusion technique which was soaked for 12h in purified water before it is used for release studies. The dialysis bag was sealed in one end, and 5ml

of drug loaded SLN was placed and sealed at another end. This is hanged and immersed in a beaker. The release studies were conducted for 12 hours (first 2hrs in 0.1N HCl and next 10Hrs in 6.8 phosphate buffer). The samples were diluted suitably and analyzed by UV-Visible spectrophotometric method. The % cumulative drug release was calculated. (Jong –Suep Baek *et al*., 2017).

Preparation of absorption-enhanced Solid Lipid Nanoparticles of GCV

The optimized SLN formulation with GCV which shown better drug release, less particle size, enough encapsulation efficiency was taken, and it is added with different amounts of absorption enhancers (Piperine and Chitosan) and subjected to *in vitro* absorption studies. (Joseph Shailender *et al*., 2017).

***Invitro* absorption studies (Continuous dissolution and absorption studies)**

These studies are also referred to as continuous dissolution and absorption studies. It consists of a single basket USP dissolution apparatus with a perfusion apparatus which consists of two tubes (Tube A & Tube B) connected together. Tube A and Tube B are having straight and bent cannulas respectively at their end. This perfusion compartment is attached with a chicken everted intestinal segment which is considered as receptor compartment. (Ekambaram P *et al*., 2011)

Isolation of Everted Chick intestine

Male**,** white leghorn chicks were bought from the local market weighing between 500gm and 600gm. On the other hand, the Krebs Ringer's solution was prepared by adding 6.3 gm of Sodium Chloride, 0.35 gm of potassium chloride, 0.14gm calcium chloride, and 0.16gm of potassium dihydrogen phosphate, 0.15 gm of magnesium sulphate, 2.1 gm of sodium carbonate and 5gm of glucose in one litre of distilled water.

For isolation of intestine, the chick is altered by making a median incision on the abdomen, and small intestine was isolated. The lumen was carefully cleared for mucous by rinsing with a pH6.8 phosphate buffer solution. A 6cm intestinal segment was removed and transferred to oxygenated Krebs Ringer's solution. It was washed with Krebs Ringer's solution. The proximal part of intestine was turned back and ligated on a glass rod, and everted sac is formed. (Rohan M. *et al*., 2016)

Papp (cm/sec) = (dQ/dt) x 1 / (60 x A x C_0)

Where dQ/dt = the amount of compound traversing through tissue in time t (min);
A = exposed area of the tissue
C_0 = Initial concentration of drug in the donor compartment.

$$\text{Enhancement ratio (ER)} = \frac{\text{Permeability coefficient of drug with enhancer}}{\text{Permeability of drug alone}}$$

The above study was performed for pure drug suspension as well as the best formulation for Tenofovir disproxil fumarate and Ganciclovir.

Histopathological Studies:

Histopathological studies for everted intestine (which is exposed to 6 hrs absorption studies) was performed by cutting down it to pieces and were flushed with normal saline and dipped in 10% neutral buffered formalin solution at the end of the experiment and the pieces were processed by paraffin technique. (Brijesh Shah *et al*., 2015) The cut pieces of 5-µm thickness were stained with Haematoxylin-Eosin method and were focused under a fluorescent microscope and observed for the damage of epithelial cells on the intestinal mucosa if any.

***In vivo* Pharmacokinetic studies for GCV**

a. Standard stock solution of GCV
About 10mg accurately weighed GCV pure drug was transferred into a 10 ml volumetric flask. To that 2-3 ml water was added to dissolve and made the volume up to the mark with water to produce a 1mg/ml of GCV. The stock solution was stored in a refrigerator at – 20^0 C ± 2^0 C until analysis.

b. Standard stock solution of internal standard
About 10mg of Paracetamol working standard was accurately weighed and transferred into a 10 ml volumetric flask. To that 3-5 ml methanol was added to dissolve and made up the volume with methanol to produce a 1mg/ml of paracetamol. The stock solution was stored in a refrigerator at –20^0 C ± 2^0 C until analysis

Selection of Detection Wavelength

a. Selection of Detection Wavelength for Ganciclovir
From the stock solution, 10 µg/ml of Ganciclovir solution was prepared in water, and the solution was scanned in the UV region of 200 - 400 nm and the UV spectrum was recorded by using UV detector.

b. Selection of Detection Wavelength for Internal Standard (Paracetamol)
From the stock solution, 10 µg/ml of Paracetamol solution was prepared in methanol, and the solution was scanned in the UV region of 200 - 400 nm and the UV spectrum was recorded by using UV detector.

Table 1: Selection of lipid

Name of the lipid	Melting point of the lipid	Drug: Lipid ratio	
		1:2	1:3
Glyceryl monostearate	55-60^{0}C	Turbid	Clear
Glyceryl distearate	52-55^{0}C	Turbid	Clear
Compritol 888	65-77^{0}C	Turbid	Clear
Stearic acid	69-70^{0}C	Not clear	Turbid

Table 2: Formulation Composition of SLNs of Ganciclovir

Code	Ganciclovir	Com-pritol 888 (Lipid)	Glyceryl di stearate (Lipid)	Glyceryl monostearate (Lipid)	Tween 80 (Surfactant)	Poloxamer 188 (Surfactant)
GSLN1	50	150	-	-	1%	-
GSLN 2	50	150	-	-	2%	-
GSLN 3	50	150	-	-	-	1%
GSLN 4	50	150	-	-	-	2%
GSLN 5	50	-	150	-	1%	-
GSLN 6	50	-	150	-	2%	-
GSLN 7	50	-	150	-	-	1%
GSLN 8	50	-	150	-	-	2%
GSLN 9	50	-	-	150	1%	-
GSLN 10	50	-	-	150	2%	-
GSLN 11	50	-	-	150	-	1%
GSLN 12	50	-	-	150	-	2%

Optimization of chromatographic conditions

Optimized Chromatographic Conditions for Tenofovir disproxil fumarate and Paracetamol

1. Stationary phase: Lunar C_{18} (250 x 4.6 mm i.d., 5µ)
2. Mobile Phase: Methanol: Phosphate buffer
3. Mobile phase ratio: 75: 25
4. Flow rate: 1 ml/min
5. Sample volume: 20 µl using Rheodyne 7725i injector
6. Detection: 250 nm
7. pH: 3
8. Data station: LC-20AD
9. Internal Standard: Paracetamol
10. Run time of drug: 5.8
11. Run time of IS: 7.4

Preparation of Mobile Phase

1.7011 gms of dihydrogen orthophosphate was added in 500 ml of Millipore water, and the pH was adjusted to 3 by using orthophosphoric acid, and the solution is filtered through 0.45µ filter paper and degassed in a sonicator.

Validation of HPLC methods

Validation is a process which involves confirmation or establishment by laboratory studies that a method/procedure/system/analyst can give the required accuracy, precision, sensitivity, ruggedness, etc. In the most basic form, validation of an analytical procedure demonstrates that the procedure developed is suitable for its intended purpose. Validation of the method was carried out after the development of the HPLC methods.

***In vivo* data analysis**

The pharmacokinetic parameters $C_{max,}$ $T_{max,}$ $AUC_{0-t,}$ $AUC_{0-\infty,}$ $t_{1/2}$ and k_{el} were determined using PK1 and PK2 solution (software) for individual drug treatments from the observed plasma concentration-time data. The measured plasma concentrations were used to calculate the area under the plasma concentration-time profile from time zero to the last concentration time point (AUC_{0-t}). The AUC(0-t) was determined by the trapezoidal method. $AUC_{0-\infty}$ was determined by the following equation:

$$AUC_{(0-\infty)} = AUC_{(0-t)} + C_{(t)} / K_{el}$$

k_{el} was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline.

Bioavailability studies

Bioavailability studies of the optimized formulations were carried out in healthy male Wistar rats between the developed solid lipid nano formulation and the pure drug suspension to prove the safety and efficacy of the developed formulation. The protocol of the study was submitted to the Institutional Animal Ethical Committee (IAEC). A reproducible analytical technique was developed for the estimation of the drugs in the plasma samples. Various pharmacokinetic parameters such as C_{max}, T_{max}, $t_{1/2, Kel,}$ AUC_{0-t} and $AUC_{0-\infty}$ were estimated. Rats

Table 5: Invitro drug release (Ganciclovir SLN)

Time (Hrs)	GSLN 1	GSLN 2	GSLN 3	GSLN 4	GSLN 5	GSLN 6
1	28±0.12	34±0.1	37±0.13	44±0.14	28±0.12	32±0.06
2	55±0.14	65±0.11	72±0.1	78.9±0.18	46±0.14	53±0.09
3	60±0.16	70±0.13	77±0.02	83.9±0.11	51±0.16	58±0.12
4	65±0.18	75±0.15	82±0.12	88.9±0.12	56±0.15	63±0.16
5	70±0.19	80±0.17	87±0.09	93.9±0.16	61±0.14	68±0.14
6	75±0.11	85±0.12	92±0.15	98.9±.2	66±0.13	73±0.11

Values represent Mean ± SD; n=3

Table 5: Invitro drug release (Ganciclovir SLN) contd...

Time (Hrs)	GSLN 7	GSLN 8	GSLN 9	GSLN 10	GSLN 11	GSLN 12
1	36±0.16	41±2.1	26±0.12	32±0.06	36±0.09	39±0.12
2	57±0.08	58±0.11	40±0.09	45±0.09	48±0.05	50±0.11
3	62±0.06	63±0.09	45±0.15	50±0.12	53±0.07	55±0.15
4	67±0.1	68±0.08	50±0.13	55±0.15	58±0.1	60±0.13
5	72±0.14	73±0.12	55±0.08	60±0.16	63±0.14	65±0.09
6	77±0.13	78±0.17	60±0.12	65±0.12	68±0.13	70±0.011

Values represent Mean ± SD; n=3

Table 7: Optimized formulation added with absorption enhancers and their respective apparent permeability constants

Optimized Formulation number	Ganciclovir (mg)	Compritol (mg)	Poloxamer 188	Chitosan (mg)	Piperine (mg)	Papp (cm/sec)
F1	50	150	2%	2	-	3.12×10^{-5}
F2	50	150	2%	4	-	5.10×10^{-5}
F3	50	150	2%	6	-	7.16×10^{-5}
F4	50	150	2%	8	-	9.02×10^{-5}
F5	50	150	2%	-	6.5	2.15×10^{-5}
F6	50	150	2%	-	7	4.60×10^{-5}
F7	50	150	2%	-	7.5	6.22×10^{-5}
F8	50	150	2%	-	8	8.13×10^{-5}

received either test or reference formulations according to their code numbers.

RESULTS AND DISCUSSION

Table 3: Particle sizes of SLNs

Formulation No.	Particle size
GSLN 4	37.2 nm
GSLN 8	271.5 nm
GSLN 12	348.8 nm

Table 4: Zeta potential of SLNs

Formulation No.	Zeta potential
GSLN 4	-18.3mV
GSLN 8	-25.0mV
GSLN 12	-2.6 mV

The preformulation studies for Ganciclovir were conducted. There is no interaction between drug and polymers for the formulation of Solid Lipid Nanoparticles.

The drug release from the formed SLNs (GSLNs) follows a biphasic release pattern. About 30-45% of the incorporated drug was released in the first two hours followed by a slower release of the drug up to 6 hours. The amount of drug released in SLN 1 – SLN 4, SLN 5 – SLN 8 and SLN 9 – SLN 12 is different. Among the first four formulations, i.e., SLN 1- SLN4, the formulation SLN 4 shown the highest release (up to 100%) which may be due to the presence of surfactant Poloxamer 188 (2%). The surfactant Poloxamer 188 shows better drug release than the tween80 (in F1&F2) due to the higher HLB value of Poloxamer 188 (greater than 24) than tween 80 (HLB 15). Further, the higher percentage of surfactant higher is the drug release.

From the result, the release pattern was best fitted for First order and Higuchi kinetic models. It may be due to hydrophilicity nature of drug as well as lipid used in SLN formulation. It also followed Higuchi's model which implies that the release is through the diffusion process. For further confirmation on release pattern, release study was performed with Korsmeyer - Peppa's kinetic model equation. It is shown that the release exponent 'n' is less than 0.45 which implies that the mechanism of release of drug from SLN was fickian diffusion.

Table 6: % Entrapment efficiency

Formulation No.	%EE
SLN1	62.1 ±0.32
SLN2	59.3 ±0.43
SLN3	68.2 ±0.26
SLN4	64.7 ±0.28
SLN5	51.3 ±0.95
SLN6	49.2 ±0.92
SLN7	57.8 ±0.43
SLN8	54.2 ± 0.53
SLN9	44.1 ± 0.23
SLN10	42.6 ± 0.31
SLN11	48.2 ± 0.63
SLN12	46.1 ± 0.87

Values represent Mean ± SD; n=3

Table 8: Mean Plasma Concentration (Ganciclovir standard and test)

Time(Hrs)	Mean Plasma Concentration	
	Pure drug	SLN
Cmax (mcg)	3.109	4.4365
Tmax (h)	2.334	2.5
AUC_{0-t}	19.72187	26.43512
k_{eli} (h-1)	0.276927	0.189693
t½ (h)	2.515151	3.659143
AUCo- α	20.39787	33.23036

The formulations SLN1 – SLN 4 shows higher entrapment efficiency which is attributed to the presence of lipid Compritol 888. It is superior to other lipids in terms of entrapment efficiency due to its relaxed nature. Further, Poloxamer 888 causes a slight increase in viscosity of the external phase thereby diffusion speed of drug is reduced towards the external phase.

The increase in the surfactant concentration shows an increase of drug in the external phase which may be due to diffusion of the drug from lipid core into the aqueous phase, leading to the reduced % entrapment efficiency.

So, among all formulations, SLN 3 shows highest % entrapment efficiency due to the presence of Poloxamer and due to the presence of less concentration (1%).

The entrapment efficiency of all the prepared formulations was studied. The %EE of first four formulations is less when compared to SLN 5 – SLN8. The reason is assumed that the presence of piperine, the lipid is unable to hold the drug in its matrix because of its nature of making more pores on any structure. Where is in the latter case they contained the chitosan which is not having any effect on entrapment of drug as like that of piperine.

The particle size and zeta potential of SLN 4 for both drugs were performed. Both show the nanoparticulate range as mentioned in results. The zeta potential values of both drugs reveal that the particles in the dispersion were in the non-aggregated state.

Transmission Electron Microscopy (TEM)

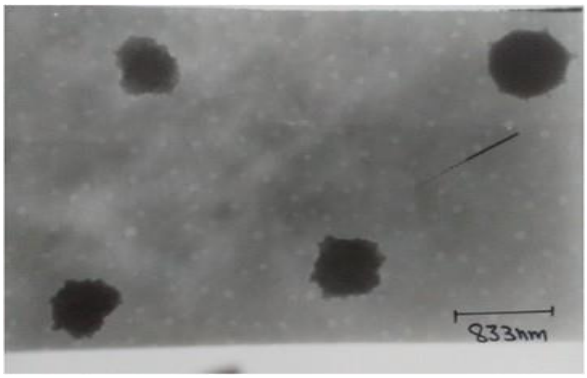

Figure 1: TEM image of GSLN 4 at 833nm

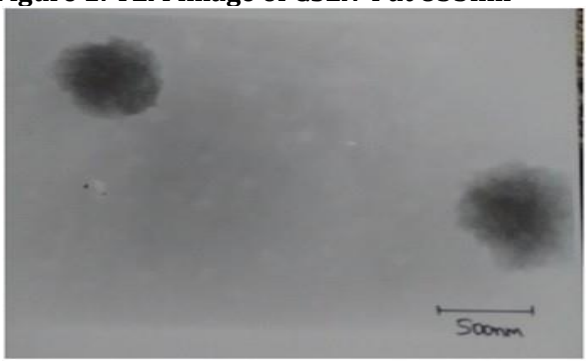

Figure 2: TEM image of GSLN 4 at 500nm

Histopathological studies

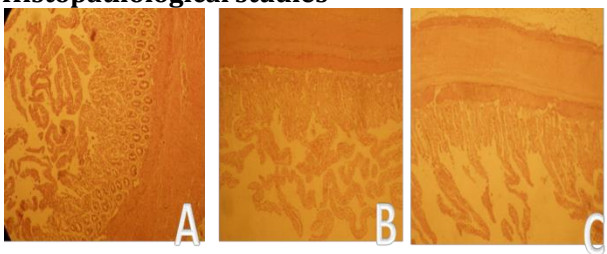

Figure 3: Histopathology of A) Control; B) F4 and C) F8

***Invivo* Pharmacokinetic studies**

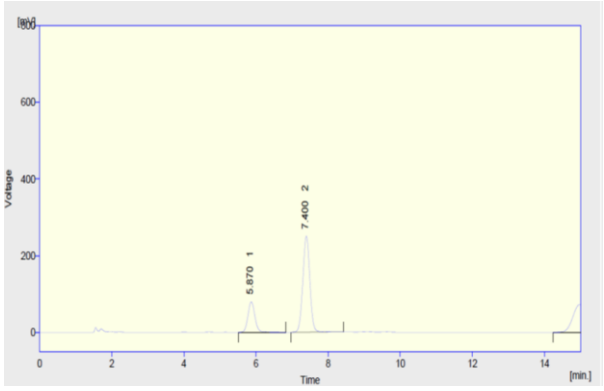

Figure 4: Typical Chromatogram of Standard

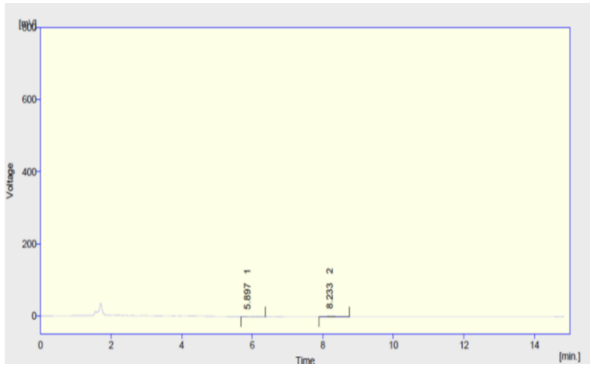

Figure 5: Typical Chromatogram of sample

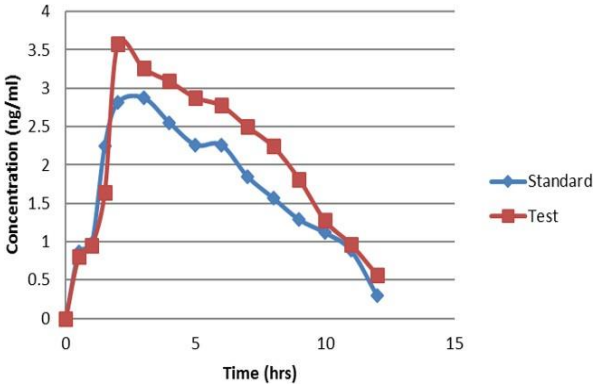

Figure 6: Ganciclovir Mean Plasma Curve

The optimized formulation of both drugs (i.e., SLN4) was taken, and it is added with different amounts of chitosan and piperine. For all the formulations of TDF and Ganciclovir (F1 – F9) absorption studies by using chicken intestine were performed by using the everted sac method. The results reveal that the permeation coefficient of F4 prepared with TDF containing 8mg of chitosan shown highest permeability coefficient (10.2 X 10^{-5} cm/sec). On the other hand, the permeation coefficient of F8 containing 8mg of piperine showed a permeability coefficient of 9.31 X 10^{-5} cm/sec which is lesser than F4. Further, the results reveal that the permeation coefficient of F4 prepared with Ganciclovir containing 8mg of chitosan shown highest permeability coefficient (9.02 X 10^{-5} cm/sec). On the other hand, the permeation coefficient of F8 containing 8mg of piperine showed a permeability coefficient of 8.13 X 10^{-5} cm/sec which is lesser than F4. So, it is clear that the bio enhancing the effect of chitosan is more when compared to Piperine.

Histopathological studies for SLN formulations with piperine, chitosan were performed. The results revealed that the formulations with piperine caused irreversible damage to epithelial cells whereas formulation with chitosan maintained epithelial cell integrity.

Pharmacokinetic studies were performed to determine different pharmacokinetic parameters of the best formulation for both drugs. All the Pharmacokinetic parameters are shown better values towards the bioavailability enhancement for the best formulation than the pure drug suspension.

Invivo Pharmacokinetics

The plasma concentrations of Ganciclovir at different points are expressed as mean, ±SD, and the mean concentration-time curve is shown in Figure 6. The calculated pharmacokinetic parameters are summarized in Table 8. The results showed that the increase in bioavailability of the developed formulation when compared to that of pure formulation and the results are summarized below. The data showed that the absorption and distribution of Ganciclovir solid lipid nanoparticle were very fast when compared to that of pure drug, with T_{max} of 2.5 hr for solid lipid nanoparticle and T_{max} of 2.33 hr for pure drug respectively. In case of mean Cmax of solid lipid nanoparticle was 4.43 mcg/ml when compared to that of the pure drug as 3.10 mcg/ml. This result revealed that increases in the concentration of drug through oral route as 0.5 folds in the formulation of solid lipid nanoparticles when compared to that of pure drug. The elimination rate for the solid lipid nanoparticle showed less with the value of 0.189 h^{-1} when compared to that of the pure drug with the value of 0.276 h^{-1} which may be due to the decrease in the particle size. The higher half-life was observed in the TDF solid lipid nanoparticle 3.65 h when compared to that of Ganciclovir pure drug 2.5h, which may be due to the lipid matrix which involves in the retaining of the drug in the drug-lipid matrix formulation. There were significant differences in AUC_{0-t} (26.43) for Ganciclovir solid lipid nanoparticle and AUC_{0-t} (19.72) for Ganciclovir pure drug, which showed that prepared formulation is more bioavailable for the systematic circulation than pure drug.

CONCLUSION

Ganciclovir is BCS Class-III drug characterized by high solubility and low permeability. Its absorption is limited by the tight junctions of epithelial cells. Absorption enhancers can overcome this sort of absorption limitations. So, Piperine and Chitosan are two natural absorption enhancers which will make the penetration of this drug through gastrointestinal epithelial cells. The target drug is formulated in the form of Solid Lipid Nanoparticles (SLNs) by the use of Piperine and Chitosan as absorption enhancers. The *exvivo* absorption studies proved that the prepared SLNs had shown better absorption than the pure drug. The SLNs prepared by Piperine showed irreversible damage to epithelial cells. Whereas the SLNs prepared by Chitosan showed reversible damage to epithelial cells which is desirable. The *in vivo* pharmacokinetic study

showed a significant increase in the Cmax, AUC, biological half-life and decrease in elimination rate constant for prepared formulation compared to pure drug

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