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Original Article

# A NOVEL APPROACH TO INCREASE THE BIOAVAILABILITY OF CANDESARTAN CILEXETIL BY PRONIOSOMAL GEL FORMULATION: *IN-VITRO* AND *IN-VIVO* EVALUATION

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**ABSTRACT**

**Objective:** The oral bioavailability of Candesartan cilexetil is less (<15%), so in this study an approach has been made to increase its bioavailability by proniosomal gel formulation.

**Methods:** The proniosomal formulation of Candesartan cilexetil was prepared by slurry method, using span 60 and Tween 60 as non-ionic surfactants, maltodextrin as carrier and cholesterol and soya lecithin as stabilizers. Prepared gel formulations were evaluated for compatibility study, entrapment efficiency, vesicle size, surface morphology, *in-vitro* diffusion studies, *in-vitro* skin permeation studies, *in-vivo* pharmacokinetics studies, various release kinetic studies and stability studies.

**Results:** FT-IR study showed no interaction between drugs and other excipients, drugs and excipients are compatible. Mean vesicles size of proniosome derived niosome was found in the range of 16.34 µm-32.48 µm and 7.25-16.45 µm before and after shaking. An optimized formulation $A_3$ containing a 2:1 ratio of span 60 and cholesterol showed maximum entrapment (86.17%) and *in-vitro* drug release (93.8%) compared to other formulations. *In-vitro* skin permeation studies were carried out using Albino rat skin and results showed that formulation $A_3$ exhibited 88.65% drug permeation in a steady-state manner over a period of 24 h with a flux value of 1.94 µg/cm$^2$/h and enhancement ratio of 3.73. *In-vivo* pharmacokinetics studies of proniosomal gel formulation $A_3$ showed a significant increase in bioavailability (1.425 folds) compared with an oral formulation of Candesartan cilexetil. Stability studies showed that proniosomal gel formulation was stable throughout its study period.

**Conclusion:** Physiochemically stable Candesartan cilexetil proniosomal gel was formulated, which could deliver significant amount of the drug across the skin in a steady-state manner for the prolong period of time in the treatment of hypertension.

**Keywords:** Bioavailability, Candesartan cilexetil, proniosomal gel, *In-vitro* diffusion studies, Entrapment efficiency, Span 60, Tween 60.



## INTRODUCTION

In recent years, many drug molecules have been discovered in the pharmaceutical field. Most of the new drug candidates used is sparingly soluble or poorly soluble and these are associated with poor bioavailability. To improve their solubility and bioavailability various formulation strategies was made; these include the use of surfactants, complexation, nanoparticles, solid dispersions, micronization and self-emulsifying drug delivery systems. Most of these approaches have shown limited success because of the need of a longer processing time, costly investments as well as complicated manufacturing process [1, 2].

In recent years, vesicular based drug delivery system such as liposomes and niosomes is developed in order to improve bioavailability of hydrophilic as well as lipophilic drug. These vesicular systems are promising systems for transdermal drug delivery as they act as vehicles or as permeation enhancers for drugs. They also possess greater advantage for a poor soluble drug by increasing solubility, controlling the release and prolonging drug activity over long periods of time. Inspite of having good chemical stability these systems are suffering from various physical stability problems such as aggregation, fusion, leakage or hydrolysis of encapsulated drugs.

In order to overcome these drawbacks, the researchers gave focus on the development of dry products. Such dry product is called as proniosomes ''dry niosomes'' and they are hydrated immediately before use, thus avoids such problems [3, 4].

Candesartan cilexetil is an esterified prodrug of Candesartan, a potent, long-acting and selective angiotensin (II) type-1 receptor antagonist used in the treatment of hypertension and congestive heart failure [5]. Candesartan cilexetil is white to off-white crystalline powder having melting point of 157-165 °C and is water insoluble. It is highly bound to plasma proteins (>99%) and does not penetrate red blood cells. It is available in 4 mg, 8 mg, 16 mg and32 mg. It can be used in the dose range of 8-32 mg/day [6].

The main drawback of this medication as an oral dosage form is its poor aqueous solubility and low oral bioavailability i.e.<15%. In the present study, an attempt was made to enhance the solubility and bioavailability of Candesartan cilexetil by transdermal proniosomal gel formulation.

## MATERIALS AND METHODS

### Material and reagents used

Candesartan cilexetil was procured from Yarrow chem products, Mumbai, India. Cholesterol, span 60, maltodextrin, soya lecithin were procured from S. D. Fine Chem. Ltd, Mumbai, India. All other reagents used were analytical grade.

### Animal used

Male albino rats weighing 180-220 g were used for skin permeation and *in-vivo* pharmacokinetics studied.

### Ethical approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Sri Adichunchanagiri College of Pharmacy, B. G. Nagara-571448, Karnataka, India. Reg. No. SACCP/IAEC/271(b)/2014-15.

Compatibility studies FT-IR spectrophotometer (Bruker IR system) was used for infra-red analysis of samples to interpret the interactions of drug with other excipients. Preparation of proniosomal gel

Proniosomal gel formulation of candesartan cilexetil was prepared by slurry method. Altogether nine formulations were prepared by using different drug to carrier ratio. The prepared formulations were dissolved in methanol: chloroform (2:1) solution and sonicate for

about 10 min. The solution was then added to a 100 ml round bottom flask containing the accurately weighed maltodextrin. The flask containing physical mixture of drug and carrier was attached to a rotary flask evaporator to evaporate solvent at 60 to 70 rpm. The temperature and pressure were maintained 45 ˚C and 600 mm of Hg respectively until the mass in the flask had become a dry as well as free flowing product. This dry powder was referred as proniosomes and stored in a tightly closed container in the refrigerator at 4˚C temperature [7]. Proniosomes were transformed into niosome by hydrating with phosphate buffer (pH 6.8) at 80 ˚C using vortex mixture for 2-3 min. The niosome was sonicated twice for 30 s using sonicator. Then these proniosome-derived niosome were used to prepare niosomal gel. For this purpose equivalent amount of niosomal suspension containing 1 g drug was centrifuged and the pellets obtained were mixed with 1% w/v carbopol 934 dispersion and made it viscous using sufficient quantity triethanolamine solution. Formulation design of Candesartan cilexetil proniosomal gel is tabulated in table no.1. This prepared niosome gel was subjected to various evaluation studies [8].

**Table 1: Composition design of candesartan cilexetil proniosomal gel**

| S. No. | Ingredients | Formulation code | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $A_1$ | $A_2$ | $A_3$ | $A_4$ | $A_5$ | $A_6$ | $A_7$ | $A_8$ | $A_9$ |
| 1. | Candesartan cilexetil (mg) | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| 2. | Maltodextrin (mg) | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| 3. | Span 60 (mg) | 100 | 150 | 200 | 100 | 100 | 100 | - | - | - |
| 4. | Tween 60 (mg) | - | - | - | - | - | - | 100 | 150 | 200 |
| 5. | Cholesterol (mg) | 100 | 100 | 100 | 150 | 200 | 250 | 100 | 100 | 100 |
| 6. | Soya lecithin | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 7. | Carbopol 934 (%) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

$A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, $A_9$= formulation code of proniosomal gel.

**Evaluation of proniosomal gel**

**pH determination**

pH of prepared gel was determined by using calibrated pH meter [9].

**Viscosity and rheological studies**

Brookfield digital viscometer (Model LVDV–E, USA) was used for the determination of viscosity and rheological properties of gel [10].

**Drug content analysis**

Proniosomes equivalent to 50 mg were taken into a standard volume flask. They were lysed with 25 ml of medium for 15 min. The clear solution was diluted to 100 ml of medium. Then 10 ml of this solution was diluted to 100 ml of phosphate buffer at pH 6.5. Aliquots were withdrawn and the absorbance was measured at 257 nm and drug content was calculated from the calibration curve [10].

**Vesicle size analysis**

Size and size distribution studies were done for niosomes obtained after hydration of proniosomal gel with agitation and without agitation. After hydration, the niosomal dispersion was observed under an optical microscope with a calibrated eyepiece micrometer which is calibrated with a stage micrometer at 40x magnification [9].

**Entrapment efficiency**

To determine loading capacity of proniosomal gels, 20 mg of Candesartan cilexetil proniosomal gel was weighed and dispersed in phosphate buffer of pH 6.5 and warmed a little for the formation of niosomes. Free Candesartan cilexetil was separated from Candesartan cilexetil loaded niosomes by centrifugation at a speed of 14000 rpm for 45 min at 4 °C. The amount of free drug in the supernatant was determined at 257 nm using UV spectrophotometer. The entrapment efficiency (EE) of Candesartan cilexetil was calculated by using following equation [11].

The entrapment efficiency was calculated by using following equation:

$$EE\ (\%) = [(C_t - C_f)/C_t] \times 100$$

Where, $C_t$ = total drug and $C_f$ = free drug.

**Vesicular morphology**

Shape and surface morphology of optimized formulation was carried out by scanning electron microscopy and photomicrography [9, 11].

***In-vitro* release studies and *in-vitro* skin permeation studies**

*In-vitro* release studies and *in-vitro* skin permeation studies of proniosomal gel were carried out for 24 h using pre-soaked cellophane membrane and Albino rat skin respectively. Both act as a receptor compartment. The proniosomal gel equivalent to 16 mg of drug was placed in a glass tube containing membrane. The glass tube was placed in a beaker containing 350 ml of phosphate buffer of pH 6.5 containing 0.35% tween 20 which acts as the receptor compartment (37±1ºC). At appropriate time intervals aliquots of 1 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analysed at 257 nm [12, 13].

**Selection of optimised formulation**

After subjecting all formulations to permeation studies, data of cumulative amount of drug permeated through the skin (mg/cm$^2$) plotted as a function of time (t). Drug flux at steady state ($J_{ss}$) was calculated by dividing the slope of the graph linear portion with the diffusion cell area (mg/cm$^2$/h). Permeability coefficient ($K_p$) was calculated by dividing drug flux at steady state ($J_{ss}$) with the initial concentration of drug in the donor compartment (cm/h) [13, 14].

***In-vivo* bioavability studies**

For *in-vivo* bioavability studies, twelve Albino rats were taken. Rats were divided into two groups (Group A and Group B) each carrying six rats. Group A was subjected to transdermal treatment with optimized proniosomal gel formulation. The rat abdominal hair was carefully shaved by a razor without any skin damage and wash with distilled water. The proniosomal gel was applied to the rat skin with the entire surface in intimate contact with the stratum corneum. In order to keep the gel secured at the site of application, the microporous adhesive tape was rolled over the gel. Group B was administered with marketed Candesartan cilexetil tablet.

The dose was calculated based on the body weight of the rats as per the surface area ratio method. The rats were anesthetized using ether. Then the blood samples (0.5 ml) were withdrawn from the tail vein of rats at 0.5, 1, 2, 3, 4, 6, 8, 12, 18 and 24 h in micro-centrifuge tubes containing the anti-coagulant (sodium citrate buffer). The blood sample was then centrifuge at 4500 rpm for 5 min in order to obtain plasma and store plasma at-20 °C before analysis. The plasma samples were deproteinised by using acetonitrile, again centrifuge and supernatant liquid was separated and finally analysed using UV spectrophotometer at 257 nm. From the results obtained various pharmacokinetics parameters such as $C_{max}$, $T_{max}$, $AUC_{0-t}$, were calculated. $C_{max}$ and $T_{max}$ were directly obtained from the graph. $K_E$ and AUC were calculated by using the residual method and trapezoidal method respectively [13, 15].

**Drug release kinetics**

An investigation of the drug release from gel was done by studying the release data with zero order, first order kinetics and Higuchi equation. The release mechanism was understood by fitting the data to Korsmeyer Peppas model [16].

**Stability studies**

The optimized formulation was subjected to three month stability studies at 25ºC/60% and 40ºC/75% RH [17].

**RESULTS AND DISCUSSION**

**Compatibility studies using FT-IR**

All the characteristic peaks of Candesartan cilexetil were present in the spectrum of drug polymer mixture, indicating compatibility between drug and polymer. The spectrum confirmed that there is no significant change in the chemical integrity of the drug. There is no change in functional group peaks (-C-H, C-N, N-H, C-O-C, C=O) of Candesartan cilexetil in all the IR-spectra and is shown in table 2 and fig. 1, 2 and 3.

**pH determination**

pH values of prepared proniosomal gel were found in the range of 6.7-7.3, which is physiologically acceptable range for topical preparations (table 3).

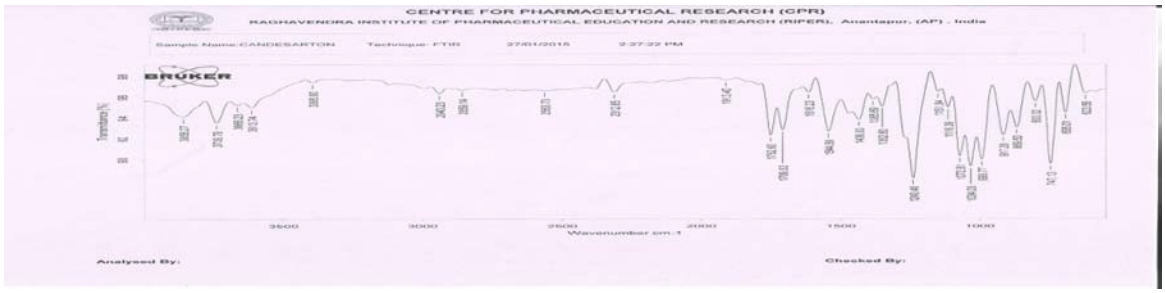


**Fig. 1: IR Spectrum of pure drug candesartan cilexetil**

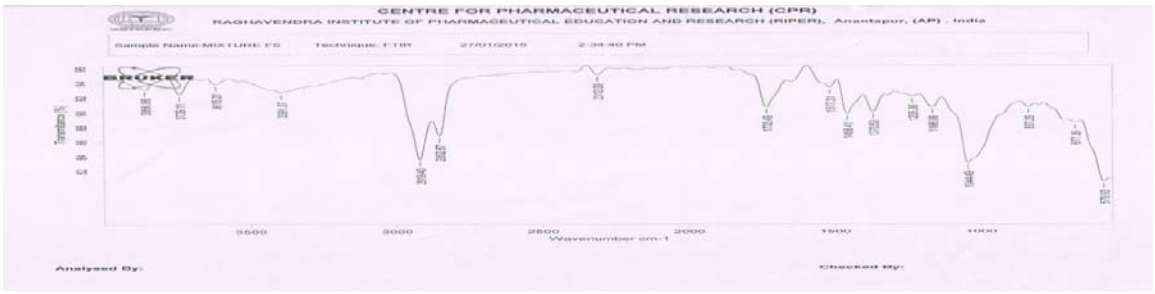


**Fig. 2: IR Spectrum of pure drug and its physical mixtures**


**Fig. 3: IR Spectrum of candesartan cilexetil proniosomal gel**

**Table 2: Interpretations of IR-spectrum pure drug, physical mixtures and gel formulation**

| Functional group | Wave number ($cm^{-1}$) | | |
|---|---|---|---|
| | Pure drug | Drug+physical mixtures | Gel formulation |
| Aromatic-C-H (s) | 2940.2 | 2919.40 | 2927.78 |
| Aromatic-C-H (b) | 747.13 | 677.35 | 928.18 |
| C-N (s) | 1116.3 | 1166.99 | 1080.07 |
| N-H (s) | 3613.7 | 3615.21 | 3737.73 |
| C=O (s) | 1752.6 | 1752.10 | 1642.86 |
| C-O-C | 1240.4 | 1235.36 | 1276.31 |

**Viscosity and rheological studies**

Viscosity of all formulations decreases with an increase in share rate, indicating pseudo plastic flow and follow non-Newtonian flow. For the topical application, the consistency of the sample is important feature, due to the fact that it must be applied to skin in the thin layer. For this reason, it is preferable to formulate non-Newtonian formulations because of their low resistance to flow when they are applied under the high shear rate. Results were tabulated in table 3 and fig. 4.

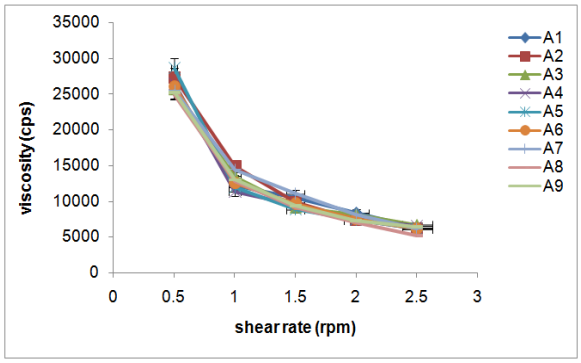


**Fig. 4: Rheological profile of candesartan cilexetil proniosomal gel formulation (A1, A2, A3, A4, A5, A6, A7, A8, A9)**

### Drug content estimation

Drug content of all proniosomal gel formulation was found between 94.65% to 98.42% w/w and a result complies with pharmacopoeial limits (table 3).

### Vesicle size analysis

Vesicle size of Candesartan cilexetil was found in the range of 7.25 µm to 16.45 µm. The results obtained from vesicle size analysis showed that the formulations ($A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$) containing span 60 as non-ionic surfactant were smaller in size than vesicles formed with tween 60 ($A_7$, $A_8$, $A_9$). Larger size vesicles formed from tween 60 might be due to lower hydrophobic nature of tween 60. The relationship observed between niosome size and span hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity, resulting in the vesicles with small size. Formulations $F_6$ containing high concentration of cholesterol also slightly reduced the vesicle size of niosomes which might be due to increasing in the hydrophobicity attributed by cholesterol. The vesicle size of niosome was larger when the dispersion was not agitated but, after agitation the size of vesicle was reduced due to breakage of larger vesicles to smaller vesicles.

### Entrapment efficiency (EE)

Entrapment efficiency of proniosomes formulations ranged from 62.70% to 86.17%. Niosomes formed from span 60 proniosomal gels exhibits higher EE than proniosomal gels prepared from tween 60 because Span 60 is solid at room temperature and have the highest phase transition. Span 60 is having the same head group with different alkyl chains. An ability to entrap drug is higher in span 60 which might be due to presence of longer saturated alkyl chain. A larger alkyl chain present in span 60 might lower the HLB value of span 60 and thus increases the EE of the drug. Formulation $A_3$ containing span 60: cholesterol in 2:1 ratio showed highest EE (86.17%). As the cholesterol content of the formulation was increased, the EE of the drug was also increased. The use of cholesterol in the proniosomal formulations not only improves the fluidity but also improves the stability of the bilayer membrane because EE of niosome is governed by the ability of formulation to retain drug molecules in the bilayer membrane of the vesicles. This characteristic of cholesterol decreases leakage of the drug molecule from the bilayer structure and also provides spherical smooth surface to the bilayer vesicles. However, further increase in cholesterol level lowers the drug EE of bilayer vesicles formulation ($A_5$, $A_6$). This could be due to the fact that the cholesterol beyond a certain level starts disrupting the regular bilayer structure of vesicles leading to loss of drug entrapment.

### Vesicle morphology

### Scanning electron microscopy (SEM) and photomicrography

SEM and photomicrography of Candesartan cilexetil proniosomal gel formulation $A_3$ revealed that proniosomal vesicles are in smaller diameter and spherical shape with a uniform surface. It is believed that vesicles with smaller diameter and spherical shape can be better diffuse through the skin as smaller vesicles tend to fuse readily. The photographs revealed that the niosomes are spherical in shape and no aggregation or agglomeration was observed.

**Table 3: Results of pH, drug content, % entrapment and vesicle size of proniosomal gel**

| Parameters | $A_1$ | $A_2$ | $A_3$ | $A_4$ | $A_5$ | $A_6$ | $A_7$ | $A_8$ | $A_9$ | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| pH* | 6.8 | 7.0 | 6.7 | 7.1 | 7.2 | 6.8 | 7.0 | 7.1 | 7.3 | 7.1 |
| Drug content (%)* | 96.7 | 95.65 | 98.42 | 96.1 | 95.1 | 97.30 | 94.65 | 97.9 | 98.1 | 97.65 |
| PercentEntrapment* | 76.8 | 83.74 | 86.8 | 82.7 | 75.49 | 74.92 | 62.7 | 66.9 | 68.4 | -- |
| Vesicle size (µm)* | 10.2 | 10.18 | 9.76 | 9.41 | 8.67 | 7.25 | 16.4 | 14.7 | 13.5 | -- |

*n =3, Average of three determinations for pH, drug content, percentage entrapment and vesicle size. µm denoted in micrometer.

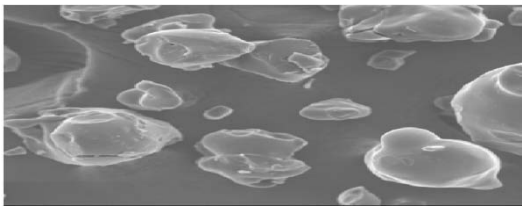

**Fig. 5: SEM images of an optimized formulation $A_3$**

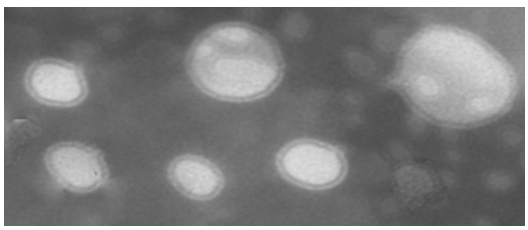

**Fig. 6: Photomicrography images of an optimized formulation $A_3$**

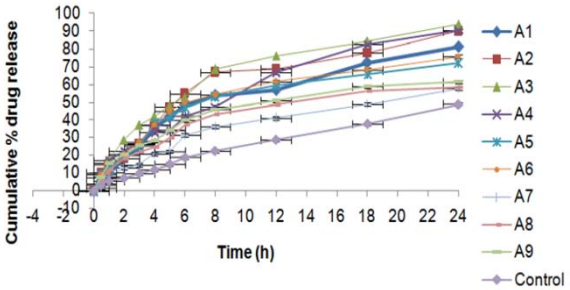


**Fig. 7: *In-vitro* drug release profile of the candesartan cilexetil proniosomal gel formulation ($A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, $A_9$)**

### *In-vitro* drug release studies

In this present study *in-vitro* release profile of Candesartan cilexetil proniosomal gel was compared with control gel of Candesartan cilexetil (normal gel). The *in-vitro* diffusion release profile of Candesartan cilexetil from proniosomes of different cholesterol

contents was apparently biphasic release process. In the first 6 h of release studies, rapid release of drug (about 31.42% to 54.66%) was observed from almost all formulations which might be due to the initial bursting of improper niosomes in the formulations (fig. 6). However, in between 6 h to 24 h, most of the formulation showed 57.62% to 93.81% of drug release. In case of control gel release rate was found relatively low i.e. 49.04% after 24 h. higher release rate of proniosomal gel compared to control gel might be due to penetration enhancement effect of non-ionic surfactants. The amount of drug released from different batches of proniosomal gel formulations was in the order of $A_3$>$A_4$>$A_2$>$A_1$>$A_6$>$A_5$>$A_9$>$A_8$>$A_7$>control.

***In-vitro* skin permeation studies**

*In-vitro* skin permeation studies were performed for Candesartan cilexetil normal gel and proniosomal gel using rat skin. From this study the cumulative amount of Candesartan cilexetil permeated per unit area across excised rat skin as the function of time, flux, permeability coefficient and enhancement ratio $E_r$, was determined. The results of permeation studies showed that formulations containing non-ionic surfactants (Tween 60 and span 60) exhibited higher percentage of drug permeation compared to control formulation (46.98%) after 24 h (fig. no. 8). Candesartan cilexetil proniosomal gel showed higher permeation because non-ionic surfactant present in these formulation acts as permeation enhancers. Among nine formulations, formulation containing span 60 showed higher skin permeation compared to formulation containing tween 60. Lower skin permeation of formulation containing tween 60 might be due to the larger size of the vesicles and less lipophilic nature of the tween, which makes more difficult for these vesicles to penetrate through the skin. Among all the formulations, formulation $A_3$ containing surfactant and cholesterol in a 2:1 ratio showed higher percentage of drug permeation 85.65%. Formulation $A_3$ showed better entrapment with optimum vesicle size, which leads to better permeation through the skin. All permeability parameters such as flux ($J_{ss}$), permeability coefficient ($P_b$), and enhancement ratio ($E_r$) were found to be higher for formulation $A_3$ (table 4) compared with other formulation. All the proniosomal gel preparations, $A_1$ to $A_9$ provided absorption of the drug through the Albino rat skin up to 24 h in a sustain manner. The mechanism of proniosomes derived niosomes to transfer the drug across the skin could be penetration enhancer effect of non-ionic surfactants. These non-ionic surfactants acts by loosening the intercellular lipid barrier of the stratum corneum resulted in higher flux of the drug. Higher the flux value, larger the drug transfers directly from vesicles to the skin.

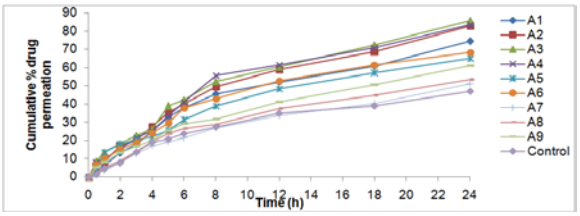


**Fig. 8: *In-vitro* drug permeation studies of proniosomal gel formulation ($A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, $A_9$, control)**

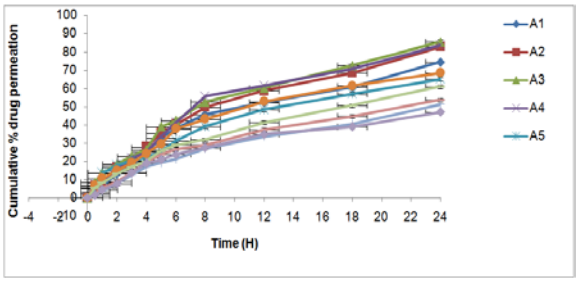


**Fig. 8: *In-vitro* drug permeation studies of proniosomal gel formulation ($A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, $A_9$, control)**

**Table 4: Permeability parameters of different proniosomal gel formulation ($A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, $A_9$ and control gel)**

| Formulation code | Flux value (J) (µg/cm²/h) | Permeability coefficient ($K_p$) (cm/h) | Enhancement ratio ($E_r$) |
|---|---|---|---|
| $A_1$ | 1.66 | 0.0835 | 3.277 |
| $A_2$ | 1.81 | 0.0910 | 3.50 |
| $A_3$ | 1.93 | 0.0970 | 3.73 |
| $A_4$ | 1.85 | 0.0925 | 3.55 |
| $A_5$ | 1.07 | 0.0535 | 2.05 |
| $A_6$ | 1.23 | 0.0615 | 2.36 |
| $A_7$ | 0.69 | 0.0345 | 1.32 |
| $A_8$ | 0.73 | 0.0364 | 1.40 |
| $A_9$ | 0.98 | 0.0495 | 1.88 |
| Control | 0.52 | 0.0225 | - |

***In-vivo* pharmacokinetics studies**

Based on the results of permeability parameters formulation A3 was selected for pharmacokinetics studies. Pharmacokinetics studies were carried out on Albino rats to evaluate the efficacy and bioavailability of the developed transdermal gel formulation against the oral dosage form. Cmax and Tmax were directly read from the graph (fig. no. 9). The mean Tmax of Candesartan cilexetil was 4 h for oral route and 6 h for transdermal route. Higher Tmax value of transdermal treatment compared to oral treatment indicated the control drug release behaviour of the transdermal gel formulation. The mean Cmax value of Candesartan cilexetil was 170.42 ng/ml for oral treatment and 164.8 ng/ml for transdermal. The Cmax values for both routes were almost similar, but graph showed that in the case of oral route the peak and valley pattern was quite evident with the fluctuation in the plasma drug concentration whereas in the case of transdermal route steady-state plasma concentration level was maintained throughout 24 h. The relative bioavailability of Candesartan cilexetil after transdermal application was found to be 1.425 times higher than that of oral delivery. The increase in bioavailability of transdermal delivery might be due to elimination of hepatic first pass metabolism by this route. In addition, transdermal gel provides much steadier plasma concentration-time curve compared to oral tablet in 24 h periods of treatment. Thus, a stable anti-hypertensive effect of Candesartan cilexetil could be obtained for longer period of time after its transdermal proniosomal gel application.

**Table 5: Pharmacokinetics parameters of Candesartan cilexetil after its transdermal and oral administration**

| Formulation | $T_{max}$ (h) | $C_{max}$ (ng/ml) | $AUC_{0-t}$ (ng. h/ml) | $K_E$ ($h^{-1}$) | $t_{1/2}$ (h) | Relative bioavailability |
|---|---|---|---|---|---|---|
| A3* | 6 | 164.8 | 1824.03 | 0.0852 | 8.133 | 1.425 |
| Marketed tablet* | 4 | 170.42 | 1298.42 | 0.0741 | 9.35 | - |

*= n, Average of three determination for each parameters

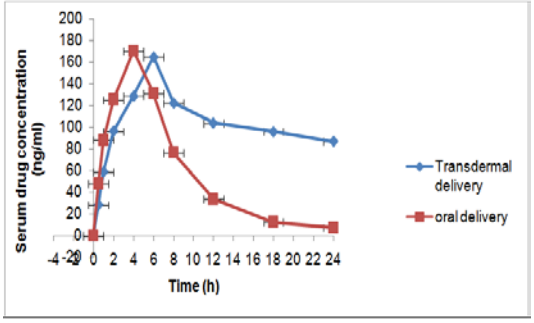


**Fig. 9: Serum concentration of candesartan cilexetil in rat serum after transdermal and oral application**

### Drug release kinetics

The cumulative release data were subjected to various kinetics models and results obtained from release kinetics studies were depicted in table no. 6. The *in-vitro* release profile of drug from all gel formulations could be expressed by Higuchi's equation, as the plots shows high linearity ($R^2$ = 0.8954-0.980) in comparison to zero order ($R^2$= 0.710-0.90) and first order ($R^2$= 0.8020-0.959). So, it was understood that Higuchi's release pattern was followed by all formulations. To confirm diffusion mechanism the data were fitted into Korsmeyer-Peppas model.

All gel formulations $A_1$ to $A_9$ showed high linearity ($R^2$ = 0.5936-0.685) with slope (n) ranging from 0.8231 to 0.905, indicating that the drug was released from proniosomal gel by diffusion-controlled mechanism. For the formulations ($A_1$, $A_2$, $A_3$, $A_7$, $A_8$ and $A_9$), the values for 'n' ranged 0.45<n = 0.89 which indicates that all these formulations followed Non-Fickian release mechanism and remaining formulations ($A_4$, $A_5$ and $A_6$) followed Super case II transport mechanism as their 'n' values are higher than 0.89.

### Stability studies

Stability studies were carried out at 25ºC/60% and 40ºC/75% RH for a period of 3 mo. Optimized gel formulations $A_3$ and $A_4$ were selected for stability studies in order to study the effect temperature and humidity on gel formulations. The gel formulation $A_3$ and $A_4$ were analysed for visual appearance, pH, viscosity, vesicle size, % entrapment efficiency, drug content and *in-vitro* release studies.

First month of stability studies revealed that there was no change in the physiochemical characteristics of both gel formulations. In between 2 to 3 mo both the formulation has shown slight changes in pH and viscosity which was in acceptable limits (±0.5). No significant changes were observed in proniosomal gel formulation during study period. Thus it can be concluded that both the formulations were stable.

**Table 6: Release exponent values and rate constant values for different formulation ($A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, $A_9$)**

| Formulation code | Kinetics models | | | | | Best fit model | Drug release mechanism |
|---|---|---|---|---|---|---|---|
| | Zero order $R^2$ | First order $R^2$ | Higuchi $R^2$ | Korsmeyer-peppas $R^2$ | N | | |
| $A_1$ | 0.7873 | 0.8987 | 0.9441 | 0.648 | 0.8845 | Higuchi | non-Fickian |
| $A_2$ | 0.7158 | 0.8026 | 0.8954 | 0.6187 | 0.8820 | Higuchi | non-Fickian |
| $A_3$ | 0.8035 | 0.9595 | 0.9623 | 0.5936 | 0.8743 | Higuchi | non-Fickian |
| $A_4$ | 0.8580 | 0.9105 | 0.9637 | 0.6560 | 0.9052 | Higuchi | Super case II |
| $A_5$ | 0.7799 | 0.8856 | 0.9397 | 0.6371 | 0.8977 | Higuchi | Super case II |
| $A_6$ | 0.7474 | 0.8347 | 0.9142 | 0.6627 | 0.9308 | Higuchi | Super case II |
| $A_7$ | 0.9053 | 0.9594 | 0.9800 | 0.6853 | 0.8231 | Higuchi | non-Fickian |
| $A_8$ | 0.8251 | 0.8993 | 0.9673 | 0.6712 | 0.8606 | Higuchi | non-Fickian |
| $A_9$ | 0.8158 | 0.9003 | 0.9689 | 0.6033 | 0.8091 | Higuchi | non-Fickian |

## CONCLUSION

In this present study, an attempt has been made to increase the bioavailability of Candesartan cilexetil by proniosomal gel formulation. From the experimental results, it can conclude that, physiochemically stable proniosomal gel was formulated and gel formulation showed 1.425 times increase in bioavailability compared to an oral dose of Candesartan cilexetil. This type formulation can deliver drug in sustain manner for the prolong period of time in the treatment of hypertension with better patient compliances and convenience.

## CONFLICT OF INTERESTS

Declared none

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