FORMULATION AND EVALUATION OF IVABRADINE HYDROCHLORIDE LOADED TRANSFERSOMAL GEL FOR TRANSDERMAL DELIVERY

GITA CHAURASIA 1, NARENDRALARIYA 2

1-2Department of Pharmaceutics, RKDF University, Bhopal, M. P. India 462033
Email: gitagec19@gmail.com

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ABSTRACT

Objective: Aim of this study was to develop the topical delivery containing ivabradine hydrochloride (IVH) loaded transpersonal gel for symptomatic treatment of chronic stable angina pectoris in coronary artery disease.

Methods: Different hydrophilic-lipophilic balance (HLB) values of surfactants-tweem-80, span-80 and sodium deoxycholate (SDC) were investigated to prepare transfersomes (TFs) respectively, with different concentration of soya phosphatidylcholine and 10% v/v ethanol in phosphate buffer solution (pH 6.8) by conventional rotary evaporation sonication method. The prepared formulations were evaluated for percentage entrapment efficiency (EE), deformability index (DI), turbidity, vesicle shape and size, in vitro drug release study and stability. SEM was done on selected formulation. Liposome formulation (LF) was prepared by using carbopol-940 as a gelling agent with propylene glycol, polyethylene glycol solution as permeation enhancer by 3-factorial design optimization methods. The developed gel was evaluated for pH, viscosity, drug content, ex vivo permeation studies and stability studies of TFs-gel. This was compared with LF-gel prepared by same procedure.

Results: Maximum % EE (78.4±6.94), suitable vesicular size (128.6 nm) and maximum DI (3.94±1.9) was found in TFs-TW-80 and selected for gel development. In vitro drug release data from TFs-TW-80, plain drug solution and liposomal formulation (LF) revealed that % cumulative drug released in TFs-TW-80 was found maximum (89.5±0.12 %) in 20 min than others. It was 2.1 times higher than LF and 3.3 times higher than the plain drug. SEM study showed spherical shape of vesicles. The drug contents in the TFs and LF gels were found to be 92 to 95% w/w. Partition coefficient for TFs-loaded gel was 1.04±0.03. Ex vivo permeation study from hairless rat skin showed that permeation of drug is described by firstly first-order kinetics than zero-order kinetics. The drug released from TFs-gel was found to be 1.7 times higher than LF-gel and about 1.9 times higher than plain drug. Flux from TFs-gel was 2.04 times greater than LF-gel and 3.28 times more than plain drug. Stability studies indicated that suitable storage condition for developed gel was temperature 25°C or less, where the pH, potency and therapeutic efficacy of formulations remain constant.

Conclusion: Thus, transdermal route has become one of the most successful and innovative focus for research in drug delivery of IVH loaded TFs-TW-80 to increase stability and bioavailability.

Keywords: Ivabradine hydrochloride, Topical delivery, Gel, Transdermal delivery, Transfersones

INTRODUCTION

IVH is an Anti-anginal drug and selective hyperpolarization-activated cyclic nucleotide-gated channels inhibitor that lowers the heart rate and used in the treatment of chronic stable angina in patients unable to take beta-adrenergic blockers and in the treatment of heart failure. IVH is water-soluble, biological half-life of about two hours, lower melting point than 200 °C and low bioavailability of 40%. Therefore, it was found a suitable candidate for transdermal drug delivery systems (TDDS). These are topically administered medicaments in the form of patches or semisolids, which can be applied to the intact skin to deliver the drug through the skin at a controlled rate to the systemic circulation. It improves therapeutic efficacy and safety of drugs [1]. In the last two decades this system has been developed in order to avoid hepatic first-pass effect and to improve drug bioavailability and to overcome the side-effects associated with oral route. The first transdermal patch was approved in 1981 by FDA [2]. In recent years, vesicular-based drug delivery system is developed in order to improve the bioavailability of hydrophilic as well as lipophilic drugs. These vesicular systems are promising systems for transdermal drug delivery as they act as vehicles or as permeation enhancers for drugs [3]. There are various strategies have been used to augment the transdermal delivery of bioactive products. The vesicular system transfersomes (TFs) loaded gel was selected for present work in comparison with liposomal gel [4]. TFs when applied to skin under suitable condition, they can transfer 0.1 mg of lipid per hour and cm2 area across. The mechanism for penetration is the generation of osmotic gradient due to evaporation of water while applying the transfersomes on the skin surface. The transport of these elastic vesicles is thus independent of concentration. The trans-epidermal hydration provides the driving forces for transport of the vesicles [5]. Elastic polymers are good candidate for topical delivery to maintain stability of drug. Carbopol-940 has excellent thickening efficiency at high viscosity at very low concentrations (less than 1%). It gives clear transparency in aqueous or hydro-alcoholic solutions suitable for use in cosmetics and topical applications. Some permeation enhancers like surfactants, polyethylene glycol (PEG), propylene glycol (PG) etc. are used. Present investigation of TFs preparation of Ivabradine hydrochloride by transdermal route is an attempt to avoid first-pass metabolism of drug and release of drug in a more controlled fashion to improve the bioavailability to stabilize the encapsulated drug.

MATERIALS AND METHODS

The sample of IVH used in this study was generously provided by Ind-swift Ltd., Chandigarh, India as a gift sample. Carbopol-940 and PG (from Himedia) Mumbai were purchased. All other chemicals and solvents used in this study were of analytical grade. Freshly distilled water was used throughout the work.

Estimation of IVH

For estimation of IVH ultraviolet (UV) spectrophotometric (UV-2400 PC series, Shimadzu, Japan) method was used, which was based on the measurement of absorbance at 286.0 nm in phosphate buffer (pH 6.8) [6]. The concentration was obtained from corresponding absorbance using calibration curve prepared in the concentration range 10-55µg/ml. The absorbance can be determined by using an equation developed by this method as y = 0.018x+0.014.

Formulation of IVH-loaded TFs

These were prepared by the conventional rotary evaporation sonication method [7]. Precisely, ratio of soya phosphatidylcholine:
membrane, J = Amount of suspension extruded in 5 min, rv measured
filter holder having 50 mm diameter by applying 2.5 bar pressure.
filter membrane (pore size diameter 100 nm), using a stainless steel
transfersomes
transfersomes vesicles w
The d
Determination of
A
Where, E = Elasticity of vesicles
Vesicles without sonication were visualized by using
M
observed visually (physical appearance).
Characterization of TFs vesicle
Turbidity measurement
10 ml of TFs from each formulation was diluted with 5 ml of distilled water and sonicated for 5 min. The turbidity was appeared and observed visually (physical appearance).
Mean vesicle size measurement
Vesicles without sonication were visualized by using an electron optical microscope (Olympus, India). A thin film of TFs was spread on a slide and a coverslip was placed over it and observed under the optical microscope using 45X lens [8].
Determination of entrapment efficiency
Vesicle preparations were kept overnight at 4 °C and ultra-
centrifuged (Remi Equipments, Mumbai, India) at 22000 rpm at a temperature of 4 °C for 2 h, whereupon the pellets of transfersomes and the supernatant containing free drug were obtained. The transfersomes pellets were washed again with distilled water to remove any entrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with phosphate buffer solution (pH 6.8) by measuring absorbance at 286 nm using UV-visible spectrophotometer (Shimadzu-1700, Japan). Same procedure was adopted with liposomal formulation. Encapsulation efficiency was calculated according to equation [9] as follows:

\[% \text{entrapment efficiency} = \frac{A_1 - A_2}{A_1} \times 100\]

Where,
A1 = Amount of drug added initially (Total drug),
A2 = Amount of drug determined in the filtrate by spectrophotometrically (Diffused drug),
(A1-A2) = represents the amount of drug entrapped in the formulation.
Determination of deformability index (DI)
The deformability study was done for all vesicular formulations against the standard liposome preparations. The elasticity of transfersomes vesicles was measured by the extrusion method. The transfersomes and liposomes formulations were extruded through filter membrane (pore size diameter 100 nm), using a stainless steel filter holder having 50 mm diameter by applying 2.5 bar pressure. The quantity of vesicles suspension, extruded in 5 min was measured [10].
E = J \times r_p^2
Where, E = Elasticity of vesicles membrane, J = Amount of suspension extruded in 5 min, r_p = Vesicles size, r_p = pore diameter.
In vitro drug release studies through dialysis membrane
On the basis of maximum % EE, suitable vesicular size and maximum DI F8 was selected for this study. Treated cellophane membrane was tied on the mouth of dialysis tube (surface area 4.52 cm²) and diffusion conditions were maintained. 50 ml of phosphate buffer (pH 6.8) was used as receptor fluid at 37 °C at 100 rpm on magnetic stirrer. Each of 10 mg IVH-loaded TFs sample from selected formulation was placed on cellophane membrane and this assembly was touched onto dissolution medium surface. Samples were withdrawn (2 ml) at regular intervals of 10 min and replaced with same amount of phosphate buffer (pH 6.8). Same procedure was adopted for ILF and plain drug (IVH). The samples were suitably diluted with buffer solution up to 3 h and absorbance was noted at 3.0 nm using phosphate buffer (pH 6.8) as blank. The percentage drug released was determined as follows:

\% \text{Drug release} = \frac{\text{released amount of drug at time } t}{\text{Initial amount of drug}} \times 100

Morphology of prepared vesicle
SEM study was carried out on selected formulation TFs-Tween-80 (F8) and on ILF.
Formulation of IVH-loaded TFs gel
The optimized TFs-Tween-80 loaded drug suspension (TFs-TW-80) was incorporated in carbopol-940 polymer with PEG-400, PG and phosphate buffer solution (pH 6.8) in optimize concentration. The formulae for preparing gel using these ingredients were optimized according to 3-factorial designs [11]. In this design, three levels of variables PEG-400 (20, 30, 40% w/w) and PG (1.5, 20, 25% w/w) were taken and the concentrations of carbopol-940 (1.5%) w/w and TFs-TW-80 suspension (1.0% w/w) were kept constant and the release rate of drug through cellophane membrane of each of nine batches was analyzed and the batch having maximum release was used for the preparation of aqueous gels. For release rate studies, phosphate buffer (pH 6.8) was prepared by I. P. (1996) method.

Rheological study of gel

The viscosities of prepared gels were measured using Brookfield Viscosimeter with spindle no. 4 (Brookfield DVII-pro viscometer), at angular velocities 32.0±0.1 °C.
Assay and content uniformity
For this study in gels (TFs and LF) was measured using spectrophotometer. Known amount of aqueous gel (500 mg) from each of formulation was dissolved separately in phosphate buffer (pH 6.8). After suitable dilution and the absorbance was measured by UV at 286.0 nm using phosphate buffer (pH 6.8) as blank. The drug content was then computed from calibration curve. Random sampling of gel at different points from the bulk was carried out for the content uniformity in gel by the above procedure [13].

Determination of partition coefficient between stratum corneum and buffer (pH 6.4)
It was carried out by mounting a known amount of the sample (1.0 g) from formulation TFs-gel, LF-gel and plain drug each, on the prepared hairless rat skin and allowed to equilibrate for 5 h. Receiver compartment was 20 ml buffer (pH 6.4). After 5 h gel was scraped off and drug content was analyzed by UV at 286.0 nm.
Amount thus partitioned into skin was calculated as partition coefficient [14].

**Ex vivo permeation study**

Drug release studies from gel is an important step during the development stages of new formulations for assuring that a drug carried by a vehicle is able to reach the skin surface at an adequate rate and in sufficient amounts. For this purpose, the release studies were performed over 48 h to be sure that the drug is released over a long period of time [15]. Male wistar rat (230g) procured from central animal house of RKDF University, Bhopal. The rat was kept under standard laboratory conditions at 25±2 °C and 55±5% relative humidity with a 12 h light/dark cycle. Rat was acclimatized for one week and placed in polypropylene cage with free access to standard laboratory diet and water. This study was conducted by following guidelines of the institutional animal ethics committee of college of pharmacy shri satya sai university of technology and medical sciences sehore, having approval number COPSS/UTMS/ANIL/19-20. The condition of the rat was monitored every day before performing the present study. Dorsal fur was removed from back portion of rat ear skin with mechanical hair clipper and depilatory cream was applied to remove small hair. The skin was immersed in distilled water maintained at 60 °C for 2 min. Fatty layers were removed and washed with saline and distilled water for use [16]. The permeation studies were carried out by using hairless rat skin. Prepared skin was tied on the mouth of dialysis tube (surface area 4.52 cm²). 50 ml phosphate buffer (pH 6.8) was used as receptor medium in a beaker on magnetic stirrer at 100 rpm at 37 °C. One gram of TFs-gel sample was placed on the rat skin and assembly was touched on the surface of dissolution medium. Sample (2 ml) were withdrawn at regular intervals of 10 min up to 4 h and replaced with same amount of buffer. These samples were diluted suitably with dilution medium. The percentage cumulative drug release was measured at 286.0 nm using UV spectrophotometer [17]. The steady state flux of IVH through rat skin was determined from the slope of the linear portion of the amount permeated per cm² (Q) versus time graph. For comparison same procedure was adopted with UF-gel and plain drug sample. All determinations were carried out in triplicate and findings were represented as statistical evaluation.

**Stability studies**

The prepared gel formulations were stored in amber colored glass vials at 4 °C, 25±2 °C and 40±2 °C for 60 days. Stability determinations were carried out in triplicate and findings were recorded by using calibrated pH-meter.

**RESULTS AND DISCUSSION**

Transfersomes were formulated using three different HLB values (hydrophilic and lipophilic) surfactants, namely SDc, tween-80 and span-80. These surfactants are biocompatible and pharmaceutically acceptable [18]. Soya phosphatidylcholine was used in different concentration as bilayer forming agent to optimize the entrapment efficiency of prepared TFs. Phosphate buffer (pH 6.8) with ethanol was used as by droting media because ethanol has a tendency to alter barrier property of the intracutaneous lipidal route for higher drug permeability [19]. Table 1 showed composition formulation of IVH-loaded TFs by using surfactants and phospholipid and their characterization properties. All formulation was found to be turbid and colloidal by observing visually due to swelling of phospholipid in hydrating medium as white dense precipitate. The suitably diluted sample of each TFs were observed optical microscopically, that showed spherical morphology of vesicles. There were significant differences in size between TFs consisting different surfactants. This size distribution was observed in range of 114.4 nm to 282.7 nm. The mean vesicular size for liposomes was 198.4 nm. In general, particle size increased along with surfactants having lower HLB value. This related with increment in the surface free energy and hydrophilicity or lipophilicity of surfactant. Surfactants interacted with lipid head groups in the membrane would increase packing density of layer, which could lead to higher surface free energy. Thus, due to fusion between lipid bi-layers by higher surface free energy leads larger size. Span-80 has strongest hydrophobicity than tween-80 and SDC, size expansion is more in span-80 TFs [20]. Among of these F8 showed maximum %EE and %DI of 78.4±0.94 and 34.9±1.9 respectively. It was observed that in F8, %EE was 2.3 times and %DI was 12.5 times greater than %DI of 78.4

| Formulation code | surfactant | Composition (SPC: SF) | Mean vesicle size (nm) | Entrapment efficiency (%EE) | Deformability index (DI) | Physical appearance |
|------------------|------------|-----------------------|------------------------|-----------------------------|------------------------|-------------------|
| F1               | TF-SDC     | 95:05                 | 170.2                  | 55.9±0.26                   | 25.3±1.8               | Turbid and colloidal |
| F2               | TF-SDC     | 90:10                 | 177.3                  | 57.1±0.17                   | 30.4±2.2               | Turbid and colloidal |
| F3               | TF-SDC     | 85:15                 | 184.8                  | 61.7±0.95                   | 31.7±2.1               | Turbid and colloidal |
| F4               | TF-SDC     | 80:20                 | 187.7                  | 60.1±1.07                   | 24.5±1.7               | Less turbid and colloidal |
| F5               | TF-SDC     | 75:25                 | 189.1                  | 53.2±0.92                   | 21.5±1.2               | Less colloidal |
| F6               | TF-TW-80   | 95:05                 | 114.4                  | 58.5±0.63                   | 22.6±1.4               | Turbid and colloidal |
| F7               | TF-TW-80   | 90:10                 | 121.9                  | 64.8±0.41                   | 31.7±1.3               | Turbid and colloidal |
| F8               | TF-TW-80   | 85:15                 | 128.6                  | 78.4±0.94                   | 34.9±1.9               | Dense and colloidal |
| F9               | TF-TW-80   | 80:20                 | 134.5                  | 76.3±1.08                   | 23.4±1.6               | Less turbid and colloidal |
| F10              | TF-TW-80   | 75:25                 | 141.8                  | 71.5±0.99                   | 10.8±0.7               | Less colloidal |
| F11              | TF-S-80    | 95:05                 | 264.9                  | 44.8±0.41                   | 12.4±1.5               | Turbid and colloidal |
| F12              | TF-S-80    | 90:10                 | 269.2                  | 45.2±0.91                   | 14.3±1.1               | Turbid and colloidal |
| F13              | TF-S-80    | 85:15                 | 273.5                  | 47.3±0.83                   | 16.8±1.6               | Dense and colloidal |
| F14              | TF-S-80    | 80:20                 | 278.3                  | 41.8±0.93                   | 09.5±1.3               | Less turbid and colloidal |
| F15              | TF-S-80    | 75:25                 | 282.7                  | 37.2±1.02                   | 05.3±0.7               | Less colloidal |
| LF               | Liposome   | (control)             |                      | 198.4                       | 34.3±1.21              | 2.8±1.3            | Turbid and colloidal |

On the basis of characterization evaluation of prepared TFs due to maximum %EE, DI and smaller mean vesicular size F8 was selected for in vitro drug release studies through the dialysis membrane. This was compared with LF and plain drug (fig. 1). Data revealed that these followed firstly first-order kinetics than zero-order kinetics. Percentage cumulative drug released in F8 was found to be maximum (89.5±0.12
%) in 20 min than others. It was 2.1 times higher than LF and 3.3 times higher than plain drug. Vesicles with smaller diameter are believed to have larger surface area to release a higher concentration of drug. Prepared TFs of tween-80 with IVH had smaller size and showed more affinity towards its hydrophilicity. SEM study of Tween-80 TFs (F8) and liposome (LF) indicated the spherical shape (fig. 2 and fig. 3).

Fig. 1: *In vitro* percentage cumulative drug release via dialysis membrane of vesicles (*mean±SD*, *n=3*)

Fig. 2: SEM study of formulation F8 (*mean±SEM*)

Fig. 3: SEM study of formulation LF (*mean±SEM*)

Thus, the selected optimized drug carrier TFs-TW-80 vesicle after characterization was used to develop gel with carbopol-940, PEG, PG, phosphate buffer (pH 6.8) in optimized concentration. The formula for the development of gel was optimized according to 3-factorial designs (table 2) and results revealed that there is maximum drug release in batch No.5 in both gel preparations (TFs and LF) after two hours. The optimized formula thus comprises of PEG-400 (30% w/w); PG (20% w/w); TFs/IF suspension (1.0% w/w); Carbopol-940 (1.5% w/w); phosphate buffer (pH 6.8) q. s. to (100.0% w/w). A second-order polynomial equation was derived from the results of all nine batches: 

\[ y = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5x_1x_2. \]

In the equation *y* is the response (drug release at 2 h). The main effect (*x_1, x_2*) represents the average results of changing one factor at a time from its low to high value. The interaction (*x_1x_2*) shows the response changes with the combined effect of factors. The coefficients corresponding to linear effects (*b_1, b_2*) interaction (*b_5*) and quadrate effect (*b_3, b_4*) were
determined from the results of the experiments (table 3). The fitted equation relating to the percent drug released in 2 h. (y) to transformed factor as follows: for LF-gel the equation is: Y(LF) = 52.5 – 6.98 x 10^-2 x1 x2 x 10^-1.62 x12 + 3.78 x 10^-2 x2 x1.04 x12 x 10^-0.008 x2 x0.25 x1 x2) x 10^-1 [22].

| Batch No. | Values for variables | % drug released within 20 min with |
|-----------|----------------------|----------------------------------|
|           | PG [% w/w] [x]       | PEG-400 [% w/w] [x]              | LF-gel | TFs-gel |
| 1         | 15 (-1)              | 20 (-1)                          | 38.3±0.04 | 41.1±0.21 |
| 2         | 15 (-1)              | 30 (0)                           | 39.8±0.11 | 45.5±0.63 |
| 3         | 15 (-1)              | 40 (1)                           | 43.1±0.08 | 48.8±0.11 |
| 4         | 20 (0)               | 20 (-1)                          | 43.4±0.31 | 52.2±0.14 |
| 5         | 20 (0)               | 30 (0)                           | 48.2±0.21 | 78.3±0.04 |
| 6         | 20 (0)               | 40 (1)                           | 42.1±0.09 | 69.5±0.06 |
| 7         | 25 (1)               | 20 (-1)                          | 37.2±1.02 | 53.3±0.22 |
| 8         | 25 (1)               | 30 (0)                           | 36.1±0.07 | 51.1±0.31 |
| 9         | 25 (1)               | 40 (1)                           | 34.5±0.02 | 47.9±0.08 |

The % drug release values are expressed as mean±SD=standard deviation from mean, n=3

| Batch No. | Values for variables | % drug released within 20 min with |
|-----------|----------------------|----------------------------------|
|           | PG [% w/w] [x]       | PEG-400 [% w/w] [x]              | LF-gel | TFs-gel |
| 1         | 25 (-1)              | 30 (-1)                          | 38.3±0.04 | 41.1±0.21 |
| 2         | 30 (0)               | 20 (0)                           | 39.8±0.11 | 45.5±0.63 |
| 3         | 40 (1)               | 15 (-1)                          | 43.1±0.08 | 48.8±0.11 |
| 4         | 20 (-1)              | 20 (0)                           | 43.4±0.31 | 52.2±0.14 |
| 5         | 30 (0)               | 20 (0)                           | 48.2±0.21 | 78.3±0.04 |
| 6         | 40 (1)               | 20 (0)                           | 42.1±0.09 | 69.5±0.06 |
| 7         | 20 (-1)              | 30 (0)                           | 37.2±1.02 | 53.3±0.22 |
| 8         | 15 (-1)              | 40 (1)                           | 36.1±0.07 | 51.1±0.31 |
| 9         | 30 (0)               | 25 (1)                           | 34.5±0.02 | 47.9±0.08 |

The % drug release values are expressed as mean±SD=standard deviation from mean, n=3

| Batch No. | Values for variables | % drug released within 20 min with |
|-----------|----------------------|----------------------------------|
|           | PG [% w/w] [x]       | PEG-400 [% w/w] [x]              | LF-gel | TFs-gel |
| 1         | 15 (-1)              | 15 (-1)                          | 38.3±0.04 | 41.1±0.21 |
| 2         | 15 (-1)              | 30 (0)                           | 39.8±0.11 | 45.5±0.63 |
| 3         | 15 (-1)              | 40 (1)                           | 43.1±0.08 | 48.8±0.11 |
| 4         | 20 (0)               | 20 (-1)                          | 43.4±0.31 | 52.2±0.14 |
| 5         | 20 (0)               | 30 (0)                           | 48.2±0.21 | 78.3±0.04 |
| 6         | 20 (0)               | 40 (1)                           | 42.1±0.09 | 69.5±0.06 |
| 7         | 25 (1)               | 20 (-1)                          | 37.2±1.02 | 53.3±0.22 |
| 8         | 25 (1)               | 30 (0)                           | 36.1±0.07 | 51.1±0.31 |
| 9         | 25 (1)               | 40 (1)                           | 34.5±0.02 | 47.9±0.08 |

The % drug release values are expressed as mean±SD=standard deviation from mean, n=3

The pH of prepared TFs-gel and LF-gel was maintained at 6.4 as per the skin pH. The rheological behavior of the gel formulation is governed by its components and its consistency. Viscosities of TFs-gel and LF-gel were found to be 11506.33±2.08 cps and 12514.72±1.81 cps, respectively.

Prepared gels were evaluated for percent drug content as per the pharmacopeial specification for topical preparation. The drug contents in the TFs and LF gels were found to be 92 to 95% w/w. The drug was found uniformly distributed in gel. The stratum corneum (SC) is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an inter-cellular lipid-rich matrix, which acts as a permeation barrier for the transdermal delivery of most drugs [23]. SC is the first and main barrier for drug penetration through skin need the partitioning of the drug. It was observed that IVH alone, TFs and LF-gel have partition coefficient (KSc/buffer) of 4.30±0.07, 1.04±0.03 and 3.11±0.21, respectively. Data suggested that the KSc/buffer had increased by dense phospholipid bilayer in lipidosome structure than IVH drug alone. Due to hydrophilicity of drug IVH, it partitioned better through skin than lipidosome. Formulation TFs-gel showed good portioning results due to the flexible behavior of its components and the deformability character of TFs.

Ex vivo permeation study from hairless rat skin revealed that the percent cumulative released of formulation TFs-gel LF-gel and IVH alone were 73.5±0.14%, 43.1±0.05% and 37.2±0.12%, respectively after twenty minutes (table 4). Fig. 4 showed that a linear relationship was obtained when the cumulative percentage release was plotted against time up to twenty minutes. This permeation of drug is described by firstly first-order kinetics than zero-order kinetics. The drug released from TFs-gel was found to be 1.7 times higher than LF-gel and about 1.9 times higher than the plain drug. The release profiles of IVH from gels showed biphasic release processes where initial burst release of the surface-adsorbed drug was observed, followed by slow diffusion from the lipid vesicles. At the initial hour, the little higher drug release of LF-gel was observed. This could be attributed to more unentrapped drug distribution in the gel system as liposomes had a lower percentage of entrapment efficiency (%EE). Unentrapped drugs could pass through the release interface earlier compared with drugs entrapped in vesicles. Afterward, lipid vesicles diffusion in gels played an important role in the release profiles and the drug release rate slowed down. TFs with higher %EE and better deformability could carry more drugs through filters quickly as compared with liposomes and plain drug. The amount of drug permeated per cm² (Q) against time revealed the flux. The slope of the linear portion of graph revealed flux up to 30 min (table 5 and fig. 5). Results concluded that the liposomes would rupture while squeezing the nano-pores, which were much smaller than vesicles sizes due to their rigid structures. They carried fewer amounts of applied drug into the deeper layer. TFs could be released from gel system and overcome the nano-porous barrier to follow the natural water gradient across the epidermis by the greater flexibility and movement of the bilayer. Then more drugs would be transferred to deeper skin [24].

| S. No. | Time (min) | Plain drug | LF-gel | TFs-gel |
|--------|------------|------------|--------|--------|
| 1      | 10         | 17.8±0.07  | 37.2±0.04 | 62.1±0.06 |
| 2      | 20         | 37.2±0.12  | 43.1±0.05 | 73.5±0.14 |
| 3      | 30         | 39.7±0.31  | 45.7±0.11 | 76.8±0.03 |
| 4      | 40         | 41.3±0.09  | 49.5±0.40 | 80.1±0.12 |
| 5      | 50         | 42.0±0.11  | 50.8±0.06 | 82.2±0.32 |
| 6      | 60         | 43.1±0.20  | 51.2±0.22 | 83.1±0.03 |
| 7      | 120        | 44.6±0.13  | 51.5±0.12 | 83.4±0.02 |
| 8      | 180        | 44.9±0.18  | 52.2±0.15 | 84.2±0.11 |

The values are expressed as mean±SD=standard deviation from mean, n=3
The storage stability testing was carried out for both gel formulations by the measurement of residual drug content, pH and physical appearance after storing at 4±2 °C and 40±2 °C after 10, 30 and 60 d. The percent residual drug content and pH of formulations were shown in table 6. There are changes in drug content over 2 mo for the stored formulations at 40±2 °C was observed. The findings of the stability study suggested that the storage of TFs at 4±2 °C and room temperature may enhance stability of transfersomes. Embedding TFs into gel may support stability of transpersonal vesicles as a result of increasing viscosity of the carrier, reducing the possibility of infusion. The pH of the formulation was found to be stable at 4±2 °C and room temperature as no changes in appearance were detected and higher temperature showed instability as hard texture was observed after 60 d. This indicated that the suitable storage condition for the formulations is temperature 25 °C or less, where the pH, potency and therapeutic efficacy of formulations remain constant.

Table 6: Stability studies of prepared gels*

| Formulation | Time (After days) | Temperature at |
|-------------|------------------|----------------|
|             |                  | (4±2) °C        | (25±2) °C       | (37±2) °C       |
| TFs-Gel     | 10               | 93±0.08         | 95.4±0.22       | 88.6±0.01       |
| (Residual drug content) | 30               | 92.7±0.19       | 93.1±0.12       | 87.4±0.27       |
|             | 60               | 92.1±0.11       | 92.5±0.07       | 86.7±0.15       |
| LF-Gel      | 10               | 75±0.06         | 79.5±0.70       | 78.7±0.81       |
| (Residual drug content) | 30               | 72.4±0.13       | 77.1±0.16       | 76.5±0.09       |
|             | 60               | 71.7±0.21       | 76.2±0.24       | 74.3±0.91       |
| TFs-Gel     | 10               | 6.4             | 6.4             | 6.5             |
| (pH)        | 30               | 6.4             | 6.4             | 6.4             |
|             | 60               | 6.4             | 6.4             | 6.3             |
| LF-Gel      | 10               | 6.4             | 6.4             | 6.5             |
| (pH)        | 20               | 6.3             | 6.4             | 6.4             |
|             | 60               | 6.2             | 6.4             | 6.3             |

*The values are expressed as mean±SD=standard deviation from mean, n=3
CONCLUSION

It was concluded that IVH-transferosomes formulated by using tween-80: soya lecithin (15: 85) showed highest %EE, highest DI, suitable vesicle size and maximum % cumulative drug release through dialysis membrane than other vesicular formulations. Evaluated IVH loaded tween-80 TFs were used to formulate gel by using carbopol-940 and permeation enhancers (PEG-400, PG) for transdermal delivery. Prepared gel was compared with liposomal gel and found that TFs are more suitable drug carrier than liposome due to its excellent deformability behavior and can transfer 0.1 mg of lipid per hour per cm² area by osmotic gradient. Stability of IVH was increased by incorporation of TFs in polymer. Thus, IVH loaded TFs are the excellent carrier for transdermal drug delivery because of topical delivery avoids hepatic first-pass effect, overcome side effects associated with oral route and improves bioavailability, safety and efficacy of encapsulated drug.

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AUTHORS CONTRIBUTIONS
All the authors contributed equally.

CONFLICT OF INTERESTS
Declared none

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