Pharmacodynamic Studies of Lercanidipine hydrochloride Transdermal Therapeutic Systems

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ABSTRACT
This article reports the assessment of lercanidipine hydrochloride (LH) for transdermal delivery. In-vitro permeation studies were carried out across the porcine ear skin in presence of various penetration enhancers. Pharmaceutical excipients and chemicals namely dimethyl sulfoxide (DMSO), isopropyl myristate (IPM), sodium lauryl sulphate (SLS), eugenol, citral, hyaluronidase were facilitated to evaluate as effective penetration enhancer. Among all, hyaluronidase emerged as effective penetration enhancer with highest flux 111.8±0.030 µg/cm²/hr, Kp 0.0172±0.040 cm/hr and enhancement ratio 5.37±0.01 than others. Later, LH (10 mg/3.14 cm²) transdermal patches (designated as EL formulations) were prepared by using EC and PVP K-30 as polymers in 1:2 ratio incorporating optimized hyaluronidase (5% w/w) as penetration enhancer, n - dibutyl phthalate (30% w/w) as a plasticizer. The ex-vivo permeation studies of EL formulations exhibited satisfactory cumulative percent of drug permeation, transdermal flux, permeability coefficient and diffusion coefficient. The curve fitment data indicated that the in-vitro permeation data of model formulations fitted well into zero order equation (average R²=0.9713 to 0.9866) better than first order and Higuchi model. The pharmacodynamic studies were carried out employing rat tail cuff method in albino rats. Hypertension was successfully induced by methyl prednisolone acetate (MPA) (40 mg/kg) subcutaneously for 2 weeks. The fabricated EL transdermal patches were significantly decreased the blood pressure (BP) in close to normal values for 24 hours. Furthermore, the satisfactory results were supported by one way ANOVA.

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INTRODUCTION
The credentials of transdermal drug delivery as an alternative and attractive route for systemic medication associated with numerous advantages. For instance, it is simple and painless, it protects the API from gastrointestinal inactivation and the influence of food, it avoids the hepatic first-pass effect, avoids the variable absorption associated with the large areas of gastrointestinal tract, effective in nauseated patients, less frequent doses and improved compliance etc. Conceivably, the major defiance for transdermal delivery is that only a limited number of drugs are tractable to administer by this route, as the outermost layer of the skin, the cornified envelope or stratum corneum, has been evolved as the principal diffusion barrier for substances, including water. To overcome this effective barrier property of skin, second generation transdermal drug delivery systems developed with the incorporation of conventional chemical enhancers like terpenes/terpenoids, anionic and non-ionic surfactants, fatty acid and esters, aprotic and its related solvents etc., or by iontophoresis or noncavitational ultrasound method. In the development of a transdermal drug delivery system, the screening of APIs for percutaneous permeation is one of the primary task in the initial design and subsequently in the evaluation of dermal or transdermal pharmaceuticals. Numerous published reports revealed that many factors can affect the transdermal permeation of a drug, including the formulation composition, nature and type of penetration enhancer, partition coefficient, source of skin etc., So it is desirable to evaluate the skin permeation characteristics of drug in-vitro before conducting in-vivo studies in human volunteers. Tactication of these physicochemical properties and the co-administration of penetration enhancers may increase transdermal delivery. Cardiovascular diseases responsible for 2.3 million deaths in India in the year 1990. The world health report 2002, presents the forecast line that it will be the largest cause of death and disability by the year 2020 in India. Hypertension is a sustained clinical condition which creates the BP values at high level. Sustained hypertension is one of the risk factors for diseases such as heart attacks, strokes, heart failure and arterial aneurysm and is the major cause of chronic renal dysfunction. Recent published data of revised WHO guidelines (Hypertension: Systolic BP $\geq$ 140 and/or Diastolic BP $\geq$ 90 mmHg) revealed a high prevalence of hypertension as 31.5 and 34 million people in rural and urban populations, respectively. Lercanidipine hydrochloride (LH), a potent antihypertensive and antianginal drug was selected as a model drug for the study. It has molecular weight 648.19 g/mol, melting point 197-201°C, pKa 6.83 at 37°C and an octanol/water partition coefficient is 6 at 20-25°C. Rationale for selection of LH, includes
complete and aberrant absorption from gastrointestinal tract after an oral dose of 10-20 mg, reduction in absolute bioavailability to approximately 10% due to extensive first pass metabolism to inactive metabolites and low half-lives of drug with 2.8 and 4.4 hours in human after single dose of 10 and 20 mg of LH respectively.\textsuperscript{20-22}

Result of literature survey and patent watch revealed that no initiation of transdermal formulation of LH in view of enhancing the bioavailability. So, the purpose of present investigation was to develop the second generation transdermal delivery system (transdermal patch) of LH by employing EC and PVP K-30, to study the effect of desired chemical penetration enhancers [dimethyl sulfoxide (DMSO), isopropyl myristate (IPM), sodium lauryl sulphate (SLS), eugenol, citral, hyaluronidase] on permeation of drug across porcine ear skin and to study the pharmacodynamic effect of LH transdermal patch on hypertension induced rats.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{lercanidipine_hcl}
\caption{Structure of Lercanidipine hydrochloride}
\end{figure}

\textbf{Materials and Methods}

\textbf{Materials}

Lercanidipine hydrochloride and hyaluronidase were procured from Leo Chem (Bangalore, India). EC (18-24 cps), polyvinyl pyrrolidone K-30, dimethyl sulfoxide, sodium lauryl sulphate, isopropyl myristate and $n$-Dibutyl phthalate were supplied by S.D. Fine Chemicals Ltd., (Mumbai, India). Eugenol and citral were procured from Tadimety aromatics Pvt Ltd, Bangalore, India. methyl prednisolone acetate was procured from local market. All remaining chemicals and solvents were reagent grade.

\textbf{Methods}

\textbf{Investigation on physicochemical compatibility of drug and polymers}

\textbf{FTIR (Fourier transformed infrared spectroscopy) analysis}

FT-IR spectroscopy (Perkin-Elmer FTIR spectrophotometer, Model 1600, Japan) was utilized as analytical tool to ascertain any interaction between LH and polymers.

An FTIR spectrum of LH was obtained using KBr disc method as per the following working condition: 5 mg of sample was triturated well with 15 mg KBr in a mortar. The triturated sample was kept in a holder, scanned in the wave length region between 400 and 4000 cm$^{-1}$ and recorded.\textsuperscript{23}

An FTIR spectrum of transdermal patch loaded with LH and polymers was obtained using NaCl plate’s method as per the following operational condition: solution of patch (triturated powder-5 mg) in a co-solvent system dichloromethane and distilled water (8:2 ml) was prepared and casted onto NaCl plates. The spectrum was recorded in the wave length region between 400 and 4000 cm$^{-1}$.\textsuperscript{24}

\textbf{DSC (Differential Scanning Calorimetry) aspects}
LH and transdermal film loaded with LH and polymers (EC and PVP K-30) were subjected to DSC (Mettler-Toledo Star System, IISc, Bangalore, India) analysis. The analysis was conducted as per the following working condition: 5 mg of drug sample was hermetically sealed in flat-bottomed aluminum pan and subjected for heating between 50-250ºC. The heating speed during the test was 10°C/minute and liquid nitrogen was used as cooling agent. The thermograms obtained for LH and transdermal film were recorded and compared.23

Ex-vivo permeation kinetic studies of lercanidipine hydrochloride with penetration enhancers

Skin preparation

In this study, porcine ear skin was used because of its close relationship in terms of histologically and biochemically to human skin25 and was found to have a closer permeability character to human skin.26,27 The fresh full thickness (75-80 µm) porcine ear skin was procured from local slaughter house. Superficial skin was excised from the back of porcine ear and hair was removed by shaving process. The cleared area was washed with PB pH 7.4. The skin was dipped in water, thermostated at 58ºC for a period of 2 min to separate the epidermis and dermal joint. The isolated epidermis (25±5 µm) was rapidly rinsed with hexane to devoid the lipid content. Next, the skin was immediately rinsed with water and then either used or stored at frozen conditions (for not more than 78 hours) in an aluminum foil for further use.28

Permeation kinetic studies

The studies were performed in triplicate. The permeation kinetic studies were conducted in Franz diffusion cell (25 ml capacity),29 consisting of donor and recipient compartments. The diameter of donor compartment provided 3.14 cm² effective constant area for diffusion studies. The excised epidermal skin was sandwiched between the donor and recipient compartments using a clamp, such that the dermis side of skin attached to donor compartment was exposed to environment and stratum corneum was facing receptor compartment. Initially, the diffusion studies were done by placing 5 ml of filtered (through Nylon membrane filter-0.22µ) saturated solution of drug in PB pH 7.4 in donor compartment. Later, studies were continued with LH solution [6.5 mg in 5 ml PB solution of pH 7.4 and constant concentration (5% v/v) of each permeation enhancer includes dimethyl sulfoxide (DMSO), eugenol, sodium lauryl sulphate, citral, isopropyl myristate and hyaluronidase individually] in the donor compartment. The receptor compartment was introduced with 25 ml of PB solution (pH 7.4) as the diffusion medium and a small teflon coated bar magnet was placed to stir the medium for uniform drug distribution. The diffusion cells were water jacketed, and controlled at a temperature of 37±1ºC by thermostatic arrangement. The entire assembly was placed on the ready to operate thermostatic controlled magnetic stirrer (Remi Motors Pvt Ltd, Vasai, India). The amount of drug permeated through the skin was determined by withdrawing aliquots of 5 ml at predetermined time.
intervals for 24 hours duration and replacing them with an equal volume of fresh PB. The drug concentration in the aliquots was estimated spectrophotometrically (Shimadzu UV-1800, Japan) at 236 nm.

**Ex-vivo skin permeation study of transdermal patches**

**Permeation studies**

The skin preparation was done as per the details mentioned in ex-vivo permeation kinetic studies of LH with penetration enhancers.

The *in-vitro* permeation studies of different transdermal patches were performed in a bi-chambered Franz diffusion cell (25 ml capacity), using a prepared porcine ear skin as *in-vitro* membrane. The skin used to simulate human derma, was tied to one end of an open-end cylinder with 3.14 cm² of diameter with stratum corneum facing it, which acted as a donor compartment. The transdermal patch was placed in donor compartment and the lower surface of skin was in intimate contact with PB solution of pH 7.4 (25 ml) placed in a receptor compartment as diffusion medium. A small teflon coated bar magnet was placed in receptor compartment to stir the medium for uniform drug distribution. The entire assembly of diffusion cell was placed on ready to operate thermostatic controlled magnetic stirrer (Remi Motors Pvt Ltd, Vasai, India). The diffusion cells were water jacketed, and maintained at a constant temperature of 37±1°C by thermostatic arrangement. At periodic time intervals, 5 ml of sample was withdrawn up to 24 hours duration and replaced with equal volume of PB. The aliquots were filtered using 0.22 µ nylon membrane filter (Millipore, India) and the amount of LH released was analyzed spectrophotometrically (Shimadzu UV-1800, Japan) at 236 nm against a reference standard using PB pH 7.4 as a reagent blank.

**Determination of flux, permeability coefficient, diffusion coefficient and enhancement ratio:**

Flux (J$_{SS}$) of drug permeated in case of *in-vitro* was calculated from slope of the steady-state portion of permeation profile by linear regression analysis. Lag time was calculated from back extrapolation. Diffusion coefficient (D/h²) and permeability coefficient (Kp) was also calculated for the *in-vitro* studies using below mentioned equations respectively,

\[
\frac{D}{h^2} = \frac{1}{6} \times T_{Lag},
\]

\[
J_{SS} = \frac{dq}{dt}.1/A,
\]

\[
K_p = J_{SS}/C_s,
\]

Where, $T_{Lag}$ is the lag time, $J_{SS}$ the flux at steady state, $C_s$ is concentration in donor compartment, $D$ is diffusion coefficient; $(dq/dt)$ is the steady state slope and $h$ is the diffusion path length.

Enhancement ratio (ER) was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by using below mentioned equation:

\[
ER = \frac{K_p \text{ of drug with penetration enhancer}}{K_p \text{ of drug without penetration enhancer}}
\]

**Fabrication of matrix type transdermal patches**

A set of initial trials were undertaken to optimize the concentration of polymer, plasticizer, volume of
casting solution, drying temperature, and drying period to prepare films of uniform thickness.

Inherently developed solvent casting technique was employed for fabrication of LH (10 mg/3.14 cm²) matrix type transdermal patches. A mixture of methanol and distilled water in a ratio of 1:1 was used as a casting solvent to prepare the EC and PVP K-30 polymeric solution in the 2:1 ratio including LH. n-dibutyl phthalate was added as a plasticizer at a concentration of 30% w/w of the dry weight of the polymers and hyaluronidase was added as penetration enhancer at a concentration of 5% w/w of the dry weight of the polymers. Accurately weighed polymers were dissolved in a beaker. The resultant homogeneous polymeric solution containing the drug, plasticizer and penetration enhancer was agitated on a magnetic stirrer (Remi Equipments Ltd., Mumbai, India) for 1 hour. The casting solution was poured on the surface of petriplate which was previously smeared with small amount of plasticizer. The solvent was allowed to evaporate at room temperature by placing inverted funnel on it to obtain the dried patch. The dried films were removed, wrapped in aluminum foil and kept in desiccators.

Data analysis
To examine the drug permeation kinetics and mechanism from the patches, the diffusion data were fitted to models representing zero-order, first-order, and Higuchi diffusion model using the below mentioned equations:

Zero order release equation
\[ Q = K_0 t, \quad (9) \]

First order release equation
\[ \log Q_t = \log Q_0 + K_t / 2.303, \quad (10) \]

Higuchi’s square root of time equation
\[ Q = K_{Ht}^{1/2}, \quad (11) \]

Whereas \( Q \) is the amount of drug release at time \( t \), \( K_0 \) is zero order release rate constant, \( K_t \) is first order release rate constant, and \( K_{Ht} \) is Higuchi square root of time release rate constant.

In-vivo studies (Pharmacodynamic studies)
Ethical approval was obtained before to commence the study from Animal Ethics Committee, St John’s Pharmacy College, Bangalore. The preclinical examination of anti-hypertensive property of the developed formulation was carried out in MPA induced hypertensive rats.

Induction of hypertension
Hypertension was induced by administering MPA subcutaneously at a concentration of 40 mg/kg/week for 2 weeks. The studies were carried out employing tail cuff method using simple, non-invasive and economic blood pressure measuring system (IITC Life Sciences, USA).

Animals
Albino rats (either sex) weighing in between 150-180 gms from Sri Venkateshwara Enterprises, Banglore, were marked distinctly with amaranth red solution for easy identification. The rats were maintained on standard rat chow with a 12 hours light/dark cycle and provided free access to food and water. Two weeks after BP was measured and twenty-four rats with the value higher than 150 mm Hg were selected for the experiment.

Measurement of Systolic Blood Pressure
Systolic BP was measured in conscious rats by the tail cuff method. All the rats were pre-conditioned to the experimental conditions before actual measurements were conducted, at the time of experiment the rats were placed in a constant temperature chamber (32°C) for 30 min. Thereafter, the animals were put in a rat holder, the tail cuff and pulse sensor was placed on the tail and connected to a rat-tail BP monitor (IITC Life science USA). The pressure in the cuff was displayed on a computer connected with BP monitor. Systolic BP was measured at the point where the reappearance of pulsations was detected by the pulse sensor, for each rat six individual readings were obtained. The highest and lowest measurements were rejected; an average of remaining was taken as the individual systolic BP.

The initial values of BP for all rats were noted down using noninvasive BP instrument. The instrument was connected to power supply and switched on. To note down the BP, the tail of the rat was first elevated by imperil it with amiable heat generated from Bunsen burner. Now, the rat was inserted into the restrainer as explained in the training period. Next the swollen tail of the animal kept in the tail cuff of the instrument and systolic BP was noted.

After recording the measured initial BP values of all rats, the rats were divided into 4 groups (designated as A, B, C and D) each with 6 rats (Table 1). Group A was considered as control (normotensive rats). Next, hypertension was instigated in the rest of the groups from B to D by s.c. injection of MPA for 2 weeks as per the method followed by Shahbaz et al. After 2 weeks duration post treatment BP values were recorded for all rats. Group B animals considered as toxic control and no further treatment with MPA. The hairs on the abdominal area of treated rats of Group C and D were removed carefully using razor. Then, placebo and LH containing transdermal patch were applied on shaven rats of Group C and D. The rat was then carefully inserted in the holder and the BP from the tail was noted up to 24 hours with 2 hours time intervals.

**Statistical Analysis of Data**
The results of evaluation of transdermal patches were subjected to analysis by one-way analysis of variance (Repeated Measures) using Graph Pad Prism software-5 version (Graph Pad software Inc., San Diego, CA, USA). Paired t-test was used to compare different formulations and p-value of < 0.001 was considered as to be significant.

**Results and Discussion**

**Investigation on physicochemical compatibility of drug and polymer**

**IR spectroscopy analysis**

Figure 2 of FTIR spectra of LH alone, exhibited principal peaks at 3078.8 cm⁻¹ for C-H aromatic stretching, 1347.03 cm⁻¹ for -NO₂, 1672.95 cm⁻¹ for >C=O stretching vibrations and 1486.85 cm⁻¹ for -CH₃ bending vibration. The obtained peaks confirm the purity and authentication of drug. On keen observation of FTIR spectra (Figure 3) of LH
Table 1: Treatment plan for various groups of animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of rats</th>
<th>Time intermission for the measurement of BP (in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>6</td>
<td>0, 2, 4, 6, 8, 10, 12, 24.</td>
</tr>
<tr>
<td>B</td>
<td>MPA (Toxic Control)</td>
<td>6</td>
<td>0, 2, 4, 6, 8, 10, 12, 24.</td>
</tr>
<tr>
<td>C</td>
<td>MPA + placebo film</td>
<td>6</td>
<td>0, 2, 4, 6, 8, 10, 12, 24.</td>
</tr>
<tr>
<td>D</td>
<td>MPA+ EL Transdermal patch</td>
<td>6</td>
<td>0, 2, 4, 6, 8, 10, 12, 24.</td>
</tr>
</tbody>
</table>

Figure 2. FTIR spectra of Lercanidipine hydrochloride

Figure 3. FTIR spectra of Lercanidipine hydrochloride transdermal patch prepared with polymers, EC and PVP-K30.

Figure 4. DSC thermogram of Lercanidipine hydrochloride

Figure 5. DSC thermogram of lercanidipine hydrochloride transdermal patch prepared with polymers, EC and PVP-K30.

Figure 6. Ex-vivo permeation kinetic studies of Lercanidipine hydrochloride with penetration enhancers (n = 3).
Table 2: Data of permeability coefficient, flux and enhancement ratio of lercanidipine hydrochloride and lercanidipine hydrochloride with permeation enhancers

<table>
<thead>
<tr>
<th>Name</th>
<th>Transdermal flux (µg/cm²/hr)</th>
<th>Permeability coefficient (cm/hr)</th>
<th>Enhancement Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>20.90±0.010</td>
<td>0.0032±0.052</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>LH+DMSO</td>
<td>93.06±0.086</td>
<td>0.0143±0.049</td>
<td>4.46±0.08</td>
</tr>
<tr>
<td>LH+Eugenol</td>
<td>91.24±0.044</td>
<td>0.0140±0.061</td>
<td>4.37±0.06</td>
</tr>
<tr>
<td>LH+Sodium lauryl sulphate</td>
<td>69.09±0.050</td>
<td>0.0106±0.046</td>
<td>3.31±0.05</td>
</tr>
<tr>
<td>LH+Citral</td>
<td>76.32±0.091</td>
<td>0.0117±0.028</td>
<td>3.65±0.02</td>
</tr>
<tr>
<td>LH+Isopropyl myristate</td>
<td>46.52±0.042</td>
<td>0.0075±0.092</td>
<td>2.34±0.05</td>
</tr>
<tr>
<td>LH+Hyaluronidase</td>
<td>111.8±0.030</td>
<td>0.0172±0.040</td>
<td>5.37±0.01</td>
</tr>
</tbody>
</table>

transdermal patch prepared with polymers EC and PVP-K30 displayed the peaks at 3073.98 cm⁻¹ for C-H aromatic stretching, 1347.03 cm⁻¹ for -NO₂ stretching, 1671.98 cm⁻¹ for >C=O stretching vibrations and 1486.85 cm⁻¹ for -CH₃ bending vibrations. However, few additional polymeric peaks were obtained in formulation.

DSC aspects

DSC aspects were also employed as a significant analytical tool to assess any interactions between the LH and polymers, EC and PVP-K30 in transdermal patch terms of their thermal behavior. The DSC thermogram (Figure 4) of drug alone displayed the characteristic endothermic peak at 196.68°C corresponding to its melting point.

The DSC analysis (Figure 5) of LH in transdermal patch, unveiled an insignificant change in the melting point (194.50°C), due to the high molecular weight of polymers.

**Ex-vivo permeation kinetic studies of lercanidipine hydrochloride with penetration enhancers**

Steady state permeation kinetic studies were performed using Franz diffusion cell, employing a porcine ear skin as in-vitro membrane using PB solution of pH 7.4 in triplicate. The results of cumulative amount of drug and drug with various enhancers permeated are displayed in Figure 6.

**Figure 7. Enhancement ratio for Lercanidipine hydrochloride with penetration enhancers (n = 3).**
Table 3: Results of cumulative percent of drug that permeated, transdermal flux, permeability coefficient and diffusion coefficient of lercanidipine hydrochloride transdermal patches.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Q_{24} (%) ±SD</th>
<th>J_{ss} (µg/cm²/h) ±SD</th>
<th>K_p (cm/hr) ±SD</th>
<th>Diffusion coefficient (cm/hr×10⁻²) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL4</td>
<td>63.90±2.1</td>
<td>86.02±1.6</td>
<td>8.60×1</td>
<td>5.575±0.1</td>
</tr>
</tbody>
</table>

Table 4: Results of curve fitting data of the in-vitro permeation data for lercanidipine hydrochloride transdermal patches

<table>
<thead>
<tr>
<th>Batch</th>
<th>Zero Order R² ± SD</th>
<th>First Order R² ± SD</th>
<th>Higuchi R² ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL</td>
<td>0.9859±0.013</td>
<td>0.8768±0.018</td>
<td>0.7795±0.020</td>
</tr>
</tbody>
</table>

Mean ±SD Standard deviation
Average of triplicate results

The effective barrier function of the skin is due to the intercellular lipids of stratum corneum and thus it is difficult for drugs to penetrate the skin. Permeation studies were carried out with 6.5 mg of drug, which is the measured quantity of saturation solubility of drug in 5 ml PB pH 7.4. Various penetration enhancers of constant concentration at 5% v/v were evaluated. All calculated results related to permeability coefficient, flux and enhancement ratios of drug are depicted in Table 2. The permeability coefficient, flux, and enhancement ratios of drug with different penetration enhancers found to be in increasing order as follows: IPM>SLS>citral>eugenol>DMSO>Hyaluronidase.

Figure 7 represents the bar graph displays the enhancement ratios of the drug and penetration enhancers.

An aprotic solvent, dimethyl sulfoxide, have shown permeation enhancement may be due to extraction of skin lipids or denaturation of stratum corneum proteins or formation of hydrogen-bonded complexes with stratum corneum lipids and the distortion. Terpenes such as eugenol and citral exhibited the mechanism of enhancement of diffusivity of the drug through the stratum corneum either by disruption of the intercellular lipid barrier or by opening polar channels in the stratum corneum through increasing electrical conductivity of tissues or by increasing the thermodynamic activity of drug. The enhancement ability of anionic surfactant such as sodium lauryl sulphate exhibited permeation due to interaction with the stratum corneum by increasing local water concentration with successive swelling and expansion of the thickness of the tissue. A fatty alcohol, IPM exhibited enhancement effect by disrupting the lipid of stratum corneum and reducing its resistance. An enzyme hyaluronidase as a permeation enhancer impressively enhanced the flux and enhancement ratio than others. This is due to either disruption of lipid organization, making the drug permeate through micro cavities and hence increasing the volume available for drug diffusion or by increasing the permeability of connective tissues and decreasing the viscosity of body fluids by transglycosidase and hydrolytic activities, respectively.
Ex-vivo skin permeation study of transdermal patch

Release pattern of the drug from the transdermal patches is majorly governed by the chemical properties of the drug and delivery dosage form, as well as physiological and physicochemical properties of biological membrane used as a barrier. An in-vitro skin permeation study is significant and essential in terms of prediction of in-vivo performance of patches and to know the rate and mechanisms of percutaneous absorption of drugs. Permeation kinetic studies were performed using a Franz diffusion cell.

Table 3 illustrates the cumulative percentage of drug permeated, transdermal flux, permeability coefficient and diffusion coefficient. From the results it can clearly indicated that batch EL displayed the highest cumulative percent (84.84±1.64%) of drug permeated after 24 hours. When the transdermal flux, permeability coefficients, and diffusion coefficient of batch EL was found to be value 114.4±3.21 µg/cm²/hr, 1.14×10⁻²±1.99 cm/hr and 8.468±0.086 cm/h×10⁻⁸ respectively. In the present the study, it was observed that formulations containing higher amount of hydrophilic polymer (PVP K-30), the drug permeation found to be increased. The controlled release of drug by the insoluble polymer (EC) was coupled with incorporation of hydrophilic polymer exhibited the highest release rate, due to the characteristic property as solubilizer to enhance dissolution of poorly soluble drugs (LH) and better penetration enhancement effect by hyaluronidase.

The curve fitment data (Table 4) indicated that the in-vitro permeation data of most of the model formulation fitted well into zero order equation (average $R^2=0.9859±0.013$) better than first order (average $R^2=0.87689±0.018$) and Higuchi model (average $R^2=0.7795±0.020$). The best-fit model was found to be zero order model as the R value of most of these formulations was found to approach unity. The results revealed that as the quantity of hydrophilic polymer with high viscosity in high concentration in EL batch formulation, permeation of drug was increased.

In-vivo studies (Pharmacodynamic studies)

Hypertension was effectively induced in the normal rats by MPA administration for a period of 2 weeks. The hypertension was retained for 72 hours after discontinuing the MPA injection with high significant difference (P < 0.001) was found in the pre- and post-treatment BP values (Table 5). This was endorsed by BP values of control and A and toxic control B groups, confirming the published data that excessive production or administration of glucocorticoid is correlated with systemic hypertension.

The applied matrix patch was revealed the decrease in BP values significantly (Repeated measures, P < 0.001) in vicinity towards normal value and it was maintained for 24 hours. This inferred that drug was diffused into systemic circulation in rats in constant fashion up to 24 hours through the best patch.

The control (A) and treatment group (D) animals exhibited comparable BP values with significant (repeated measures, P < 0.001) difference. The
percentage of drop in mean systolic BP of rats by LH patch and placebo was 31.19% (53.31 mm Hg) and 0.57%, respectively.
Transdermal formulation (EL) successfully diminished the hypertension witnessed with relapsing the rat BP to normal condition. The BP reducing efficacy of the formulation (EL) has been presented in Figure 8.

**CONCLUSION**

On the basis of obtained statistical data and results, it was concluded that the developed matrix type transdermal patch (EL formulation) containing lercanidipine hydrochloride was fabricated successfully, which can be used for the treatment of hypertension for 24 hours. The preparation didn’t exhibit any chemical interaction. It imparted permeation of drug in viable animals (Albino rats) in a steady state fashion over a desired period of time. Future prospect is to know the further efficacy of formulation EL for *in-vivo* studies in humans.

**Declaration of conflicts of interest**

The authors declares that no conflicts of interest. Research team is alone accountable for the content and writing of this data.

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