INTRODUCTION

Transdermal drug delivery system, a topic of increasing interest to researchers, is the delivery of a therapeutic agent through the intact skin for the exertion of its systemic effect. The drugs having short biological half-lives can be administered through this route as they can avoid first pass metabolism.[1,2] For the effective permeation of drugs through the skin, various penetration enhancers are incorporated in the formulation along with the therapeutic agent for improving the permeation through the skin. Some of the penetration enhancers possess the potential to cause skin irritation. Hence, preferably those enhancers which have been listed under generally recognized as safe agents by U.S.A food and drug administration (e.g., terpenes and essential oils) must be used for transdermal applications.[3]

Ketorolac tromethamine (KT), a pyrrolizine carboxylic acid derivative, is being used as a potent nonnarcotic analgesic in the treatment of moderate to severe postoperative pain.[4] It has been included in the treatment regimen of pain associated with abdominal, gynecologic, orthopedic and urologic surgery.[5] Ketorolac tromethamine demonstrates an oral bioavailability of 90% with a very low first pass metabolism. However, upon oral administration, it exhibits adverse effects such as gastrointestinal ulceration and has a short biological half-life (4-6 h). In order to avert these adverse effects, a transdermal drug delivery system has been explored.[6]

In the present investigation, 20 penetration enhancers belonging to ten different chemical classes were selected and analyzed for the permeation enhancement of the drug KT using ex-vivo diffusion study through the rat skin.

Large number of penetration enhancers were judiciously selected for the present study to represent diverse chemical structures
Kumar, et al.: Permeation enhancement of ketorolac tromethamine

(such as chain length, polarity, level of unsaturation and presence of some groups such as alcohols, sulfur, ketones, esters, amide), as the interaction between enhancers and stratum corneum (SC) may differ resulting in variable efficiency as permeation enhancers.

MATERIALS AND METHODS

Materials
Ketorolac tromethamine was generously gifted by Ranbaxy Laboratories Ltd. (Gurgaon, India). $\alpha$-pinene, l-limonene, and fenchone were purchased from Sigma Chemical Company (St. Louis, USA). Propylene glycol (PG), acetone, dimethyl sulfoxide, dimethyl formamide, ascorbic acid, citric acid, isopropyl myristate, tweens (20, 80) and spans (20, 40, 80), and triton X-100 were obtained from Hi-Media, Mumbai, India. All other chemicals utilized were of suitable analytical grade.

Animals
Wistar rats (200-250 g) were procured from the disease-free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The experimental protocol was approved by the Institutional Animals Ethics Committee (registration number 0436).

Methods

Preparation of vehicles
The vehicles were prepared by dissolving appropriate amounts of the drug and the enhancers directly in PG. PG was selected so that both hydrophilic and hydrophobic adjuvant could be dissolved. The arbitrary concentration of enhancers was 10% (w/v or v/v). A control PG vehicle containing 1% KT and no penetration enhancer was used as a reference.

Calculation of permeability coefficient
To calculate the permeation parameters, a graph of penetrated amount of the drug versus time was plotted. The steady state flux (Jss) was calculated from the slope of the graph.

The cumulative drug permeation ($Q_t$) was calculated using the following equation:

$$Q_t = V_r C_t + \sum_{i=1}^{n} \frac{V_s C_i}{V_r}$$

Where $C_t$ is the drug concentration of the receiver solution at each sampling time, $C_i$ is the drug concentration of the $i$th sample, and $V_r$ and $V_s$ are the volumes of the receiver solution and the sample, respectively.

Data were expressed as the cumulative $Q_t$/unit of skin surface area, $Q_t$/S ($S = 1.12 \text{ cm}^2$). The Jss were calculated by linear regression interpolation of the experimental data at a steady state:

$$J_{ss} = \frac{\Delta Q_t}{(\Delta t \times S)}$$

Apparent permeability coefficients ($K_p$) were calculated according to the equation:

$$K_p = \frac{J_{ss}}{C_d}$$

Where $C_d$ is the drug concentration in the donor compartment.

The enhancement ratio (ER) was calculated from following equation:

$$ER = \frac{K_p \text{ with enhancer}}{K_p \text{ without enhancer}}$$

Statistical analysis
The statistical analysis was performed using the software GraphPad Instat (DataSet3.ISD). The results were analyzed using one-way ANOVA and then by Dunnett’s $t$-test. $P < 0.001$ was considered as statistically significant.

RESULTS AND DISCUSSION

The flux ($J$), permeability coefficient ($K_p$), and ER for each of the different enhancers obtained through studies of drug solution with concentration (1 mg/mL) through rat skin and calculated according to equations (1), (2), (3), and (4) have been tabulated in Table 1.
The permeation rate of the KT showed enhanced permeation with fatty acids. Among the saturated fatty acids, with stearic acid, the permeability flux (J) was $507.73 \pm 2.15 \mu g cm^{-2} h^{-1}$ which was 14.50-fold more than that of KT without a permeation enhancer ($71.47 \pm 0.625 \mu g cm^{-2} h^{-1}$). The addition of the unsaturated fatty acids, oleic acid (C18:1) exhibited the maximum permeation rate ($514.43 \pm 2.50 \mu g cm^{-2} h^{-1}$) as compared to the presence of the corresponding saturated fatty acid. Unsaturated fatty acid like oleic acid (C18:1) exhibited maximum permeation (J = $514.43 \pm 2.50 \mu g cm^{-2} h^{-1}$).

On the other hand, terpenes (l-1imonene, α-pinene, l-menthol, and fenchone) resulted in a 5 to 7-fold increase in permeability coefficient.

In the case of nonionic surfactants that is, tweens (20, 80) and spans (20, 40, 80), tween 80 demonstrated the highest permeation rate. The structure of tweens and spans is relevant to this role. The structure imparts both lipophilic and hydrophilic characteristics to the enhancer, allowing it to partition between lipophilic mortar substance and the hydrophilic protein domains. Nonionic surfactant increases rate of transport by two possible mechanisms, one by penetration into the intercellular regions of SC by solubilizing lipid component, thereby increasing the fluidity, secondly disruption of comeocyte by interaction and binding with keratin filament.[9,10]

The flux of KT was significantly higher in the presence of DMSO, triton X-100, and isopropyl myristate (IPM), when compared with the control. The effect of DMSO on skin permeation can be explained as the consequence of denaturation of proteins and perturbation of the bilayer lipid layer packing in the keratinized SC layer.[11]

Acetone and DMF also increased the permeation rate, but ascorbic acid and citric acid did not show any increase in permeability of drug. The aprotic penetration enhancer, DMF, probably forms solvation shell formation surrounding the polar head groups of the lipid and hence increases the permeation.[12]

Figure 1 indicates the permeation profile of all the penetration enhancers used in the present study, wherein, it can be observed that oleic acid exhibits the maximum permeation rate.

Isopropyl myristate was able to show a high enhancing effect, due to very effective penetration through nonpolar route in the skin where they disrupt lipophilic permeation.

Urea increases the permeability coefficient of KT by altering the barrier function of the skin through the increase of the hydration of the SC and inducement of keratolysis of skin after prolonged contact.[14]

Fatty acids are the most attractive skin permeation enhancers having lipophilic properties; many studies have shown that the skin permeability enhancing property increases with PG vehicles.[15] The solubility of KT in PG remained unaffected by adding the fatty acid. The increase in the permeation rate of KT with fatty acids plausibly resulted in the increased permeation coefficient. The double bonds present in unsaturated fatty acids, like oleic acid, alter the lipid structure of the skin by producing “kink,” isomer, which facilitates the permeation of drug across the skin.[16,17]

The enhancing activity of terpenes, which presumably act by reversibly modifying the barrier properties of the SC and by increasing the drug partitioning into the skin barrier, has been reported in the case of several model penetrants, such as estradiol,[18] 5-fluorouracil,[19] and indomethacin.[20]

**CONCLUSION**

It can thus be concluded that out of the 20 penetration enhancers, studied for the flux, permeability coefficient and ER, unsaturated fatty acid (oleic acid) increased the permeation rate of KT more than the saturated fatty acids (stearic and palmitic acid). Terpenes and nonionic surfactants also increased the permeation by penetrating into the intercellular regions of SC. DMSO, triton X-100, and IPM increased the ER to an extent of 4-5 folds. Acetone, urea, and DMF also increased the permeability of KT by increasing the hydration of the SC and then causing keratolysis of skin. Ascorbic acid and citric acid did not increase the

**Table 1**: Kp, flux (J), and ER in the absence and presence of the enhancers at the concentration of 1 mg/mL

| Penetration enhancers | J (μgcm⁻² h⁻¹) | Kp (cm·h⁻¹) | ER |
|-----------------------|---------------|-------------|----|
| Control               | 71.47±0.625   | 0.0350±0.00035 | 1  |
| α-Piine               | 199.70±2.23   | 0.1997±0.00223 | 5.70|
| l-Menthol             | 212.63±3.80   | 0.2126±0.00380 | 6.07|
| Span 80               | 379.93±5.61   | 0.3799±0.00561 | 10.85|
| Span 40               | 305.13±3.32   | 0.3051±0.00332 | 8.71|
| Stearic acid          | 507.73±2.15   | 0.5077±0.00215 | 14.50|
| Isopropyl myristate   | 440.93±5.30   | 0.4409±0.00530 | 12.59|
| DMSO                  | 153.03±1.55   | 0.1530±0.00155 | 4.37|
| DMF                   | 99.63±1.26    | 0.0996±0.00126 | 2.84|
| Triton X-100          | 152.23±2.81   | 0.1522±0.00281 | 4.34|
| Citric acid           | 17.92±1.09    | 0.0179±0.00109 | 0.51|
| Span 20               | 236.66±6.31   | 0.2366±0.00631 | 6.76|
| Fenchone              | 206.93±6.43   | 0.2069±0.00643 | 5.91|
| Oleic acid            | 514.43±2.50   | 0.5144±0.00250 | 14.69|
| Ascorbic acid         | 12.50±0.79    | 0.0125±0.00079 | 0.35|
| Palmitic acid         | 474.33±1.15   | 0.4743±0.00115 | 13.55|
| Tween 80              | 411.30±1.50   | 0.4113±0.00150 | 11.75|
| Urea                  | 154.06±2.50   | 0.1540±0.00250 | 4.40|
| l-1imonene            | 235.99±3.19   | 0.2359±0.00319 | 6.73|
| Acetone               | 75.59±1.72    | 0.0755±0.00172 | 2.15|
| Tween 20              | 146.23±0.94   | 0.1462±0.00094 | 4.17|

ER: Enhancer ratio, Kp: Permeability coefficient
Kumar, et al.: Permeation enhancement of ketorolac tromethamine

permeation rate of the drug. Thus, the present study may provide a platform for researchers to optimize the penetration enhancement of different penetration enhancers for the transdermal delivery of KT.

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