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Basil Oil is a Promising Skin Penetration Enhancer for Transdermal Delivery of Labetolol Hydrochloride

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The present work investigates effectiveness of basil oil, a volatile oil containing alcoholic terpenes, as a potential penetration enhancer for improved skin permeation of labetolol hydrochloride (LHCl) with reference to camphor, geraniol, thymol, and clove oil. Saturation solubilities of LHCl were determined in water, vehicle (ethanol:water, 60:40 v/v) and vehicle containing 5% w/v terpenes. Comparable ($P > 0.05$) saturation solubilities were found suggesting an insignificant increase in LHCl flux across rat skin on account of thermodynamic activity. Permeation of LHCl in vehicle per se and in presence of 5% w/v enhancer was investigated by performing in vitro rat abdominal skin permeation studies using a side-by-side glass diffusion cell. Various parameters viz. steady state flux, permeability coefficient, lag time, partition coefficient, diffusion coefficient, and enhancement ratios (ER) were calculated from the permeation data. Basil oil produced the maximum enhancement (ER = 46.52) over neat vehicle, among all enhancers. Activation energies for LHCl permeation in water, vehicle per se and in presence of 5% w/v basil oil were found to be 23.16, 18.71, and 10.98 kcal/mole, respectively. Lowering of activation energy in presence of basil oil suggests creation of new polar pathways in the skin for enhanced permeation of LHCl. Basil oil is proposed as a promising penetration enhancer for improved transdermal drug delivery of labetolol.

Keywords basil oil; labetolol; transdermal; penetration enhancer; terpenes

INTRODUCTION

Hypertension is cited as the leading cause of non-communicable disease mortality worldwide (Yach, 2002). In a multicentred study conducted by the World Health Organization (WHO) in India and Bangladesh on 1,203 participants, it was found that the prevalence of hypertension was 65%. It also was reported in the same study that only 45% of the participants, were taking oral medication and only 10% of them were fully benefiting from their medication (Hypertension study group, 2001). Reasons for this may be patient noncompliance and fluctuations in the plasma drug concentration due to an uncontrolled release of the drug from conventional dosage forms. These findings suggest that despite the availability of a plethora of therapeutically effective antihypertensive molecules, inadequate patient welfare is observed; this arguably presents an opportunity to deliver antihypertensive agents through a different route. The transdermal route has been utilized since ancient times to transport a range of therapeutically active molecules into the systemic circulation.

The major barrier to the percutaneous transport of drugs across the skin is stratum corneum, the uppermost layer of the skin (Naik, Kalia, & Guy, 2000). Several strategies have been employed to circumvent this natural barrier due to the great advantages this routes offers. One of the most widely used strategies employs the use of penetration enhancers, molecules that reduce the barrier properties of skin by acting on the different components of skin such as lipids and proteins (Williams & Barry, 2004). One promising group of candidates to be employed as clinically acceptable enhancers are terpenes. They are reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy (Okabe, Obata, Takayama, & Nagai, 1990,). Chemically, terpenes are a series of compounds consisting of isoprene (C_5H_8) units that have been shown to increase the percutaneous absorption of both hydrophilic and lipophilic molecules (Godwin & Michniak, 1999; Mohimi, Williams, & Barry, 1997).

Labetolol hydrochloride (LHCl), a potent hypotensive agent, is a combined alpha and beta blocker that acts faster than pure beta blockers (Sweetman, 2002). Oral therapy of LHCl is restricted to moderate to severe hypertension and hypertension that others drugs have failed to treat. However, oral therapy presents some problems. First, frequent administration (i.e., twice a day) can lead to less patient adherence and, second, oral therapy provides poor bioavailability (35%) due to high first pass hepatic metabolism (Genarro, 1995). Intersubject variability of LHCl in the extent of first pass metabolism is pronounced and accounts for large differences in the area under the curve (plasma concentration v/s time) observed (McNeil et al., 1979). Also, with the oral therapy of LHCl, FDA has received 11 reports of hepatocellular damage (Harvengt, 1991). However, these problems can be overcome

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by delivering LHCl transdermally as it satisfies many criteria (Dollery, 1999; Moffat, 1986; Sweetman, 2002) for a molecule to be delivered via skin. For example, LHCl is clinically effective, undergoes high first metabolism, has a favourable partition coefficient (7.08), and a lower melting point (180ºC), suggesting that it is a good candidate for delivery via transdermal route.

The present study evaluates the effectiveness of basil oil as a skin penetration enhancer for LHCl. Camphor, geraniol, thymol, and clove oil have been used as reference terpenes and volatile oil. Basil oil has been selected as the test enhancer as it is an inexpensive and rich source of terpenes particularly in this part of the world. The mechanism by which the basil oil alters the permeation of LHCL is also investigated. The separation and individual contribution of various constituents of basil oil on its overall skin enhancement capacity was beyond the scope of the present study.

MATERIALS AND METHODS

Materials

LHCl was supplied as gift sample by Neuland Labs, Hyderabad, India. Ethanol (EtOH), camphor, geraniol, and ammonium phospate were purchased from Aldrich (USA) chemicals. Clove oil was supplied by Sigma chemicals (USA). Basil oil was obtained from Alchemy chemicals (M.P., India). Thymol was supplied by Seema International (Delhi, India). Methanol HPLC grade and water HPLC grade were purchased from S.D. Fine chemicals (Delhi, India). All the reagents used were of analytical grade. Double distilled water was used in all experiments.

Animals

Male wistar rats (8 weeks old, 200–250 g) were supplied by Central Animal House Facility of Jamia Hamdard and kept under standard laboratory conditions in 12 hr light/dark cycle at 25 + 2°C. Animals were provided with pellet diet (Lipton, India) and water ad libitum. The animals were procured after the approval of the study by the Hamdard University Animal Ethics Committee (Project proposal no. 166/2004).

Analytical Methodology

Samples from solubility and permeation studies were analysed by an HPLC method reported by Muir and Ismael (1984). Briefly, the HPLC system (Shimadzu, Japan) with UV detector was used. Mobile phase consisted of methanol: 0.01 M ammonium phosphate solution (1:1) adjusted to pH 3.0 was used. Volume of sample injected was 50 µl. Mobile phase was run at a flow rate 1ml/min through a reverse phase C-8 column (HxSil, 150 × 4.6 mm, 5 µm, Hamilton Company, USA) and detection was made at 216 nm.

Solubility Studies

An excess amount of LHCl was added to water, vehicle (EtOH:water, 60:40 v/v) and vehicle system containing 5% terpenes and shaken at 32 ± 0.5ºC for more than 48 hrs. The solutions were then centrifuged at 10,000 rpm for 5 min and the supernatant was then assayed by HPLC after appropriate dilutions.

Preparation of Full Thickness Rat Abdominal Skin

The animals used for the preparation of skin were male albino wistar rats weighing between 200–250 g. The rats were sacrificed by giving excess ether anesthesia. The hairs from the abdominal surface of the rat (Krishnaiah, Satyanarayana, & Bhasker, 2003) were removed by a clipper and full thickness skin was surgically removed from the rats. The subcutaneous tissue adhering to the skin was separated with help of scalpels and the dermis side was wiped with isopropyl alcohol to remove the residual adhering fat. The skin was washed with distilled water, wrapped in aluminum foil, and stored in a deep freezer at −20ºC until use.

In Vitro Skin Permeation Studies

Transdermal permeation of LHCl across full thickness rat skin in vitro was studied using side-by-side glass diffusion assembly (Park et al., 2001), fabricated by a local glass works company, for 24 hrs. The diffusion assembly consisted of donor and receiver chambers each of capacity 10 ml with a diffusional area of 3.087 cm^2. The stratum corneum faced the donor chamber (maintained at 32 ± 0.5ºC) filled with the solution of drug (2 mg/ml) and penetration enhancer in vehicle (EtOH:water, 60:40 v/v) system. EtOH:Water (60:40) was used for the purpose of dissolving penetration enhancers in concentration of 5%. Different penetration enhancers studied were camphor, basil oil, geraniol, oil of cloves, and thymol, all containing terpenes. The receiver phase was isotonic phosphate buffer (IPB) pH 7.4 at 32 ± 0.5ºC (Buyuktimkin, Buyuktimkin, & Rytting, 1993; Vaddi et al., 2002). Samples were taken from the receiver phase at appropriate times and replaced by an equal volume of buffer. LHCl concentrations were determined by the HPLC method given under analytical methodology. Sample volume was immediately replaced with fresh receptor medium (maintained at 32 ± 0.5ºC) after each sampling.

Determination of Activation Energy

To determine activation energy, permeation studies in water, vehicle system, and 5%w/v basil oil in vehicle were carried out at different temperatures viz. 27, 32, 37, 42, and 47ºC. Permeability coefficients were calculated at each temperature, and activation energies for LHCl were determined using Arrhenius relationship.

Data Analysis

The cumulative amount of drug permeated though a unit area was plotted as a function of time. The steady state flux (J) was

determined as the slope obtained by regression of linear portion of the plot whereas the x, the intercept represented the Lag time (T). The diffusion coefficient (D_c), permeability coefficient (K_p), and partition coefficient (P) were determined from the donor phase concentration (C = 2000 µg/ml), the thickness of the barrier (e=0.15 cm), the lag time, and steady state flux (Ropke et al., 2002). The permeability coefficient was obtained by dividing J by C; diffusion is expressed by $1/6e^2\ T^{-1}$; and the partition coefficient (P) is denoted by ePD^{-1}. Enhancement ratios can be expressed as quotient of K_p in presence of enhancer and K_p in vehicle per se (control) as ER_{veh} and quotient of K_p in presence of enhancer and K_p in water per se as ER_{water}.

The activation energy was calculated from the following Arrhenius relationship:

$$\log K_p = E_{act}/2.303RT + \log K_{po}$$

where E_{act} is the activation energy, R is a gas constant, T is the temperature, and K_{po} is the Arrhenius constant.

One way analysis (Tukey test) was applied at significance level of $P < 0.05$ to analyze the data of this investigation.

RESULTS AND DISCUSSION

Saturation solubilities for LHCl in water, vehicle (EtOH: water, 60:40 v/v) per se vehicle containing 5% penetration enhancers were determined and the results are shown in Table 1. No significant differences were observed ($P < 0.05$) for LHCl saturation solubilities in vehicle, in presence and absence of 5% w/v terpenes as penetration enhancer.

Permeation profiles for LHCl across rat abdominal skin in water, vehicle per se, and vehicle containing 5% enhancers are shown in Figure 1. Different parameters viz. flux, permeability coefficient, diffusion coefficient, partition coefficient, enhancement ratio (ER_{veh} and ER_{water}), and lag time were calculated from the permeation data and the values are shown in Table 1. Among the different enhancers evaluated, basil oil was found the best enhancer as there was maximum permeation in its presence.

Activation energies of LHCl using Arrhenius relationship in water, vehicle per se, and vehicle containing 5% basil oil were found to be 23.16, 18.71, and 10.98 kcal/mole, respectively.

Effect of Terpenes on Thermodynamic Activity

Saturation solubilities of LHCl in water, vehicle, and vehicle containing 5% terpenes were determined and results are shown in Table 1. As evident from Table 1, saturation solubility of LHCl in vehicle, with and without the presence of 5% w/v enhancers is comparable ($P > 0.05$). This data suggest that terpenes did not produce any significant effect on the thermodynamic activity of molecule. Similar to the findings of this study, it was reported by Okabe et al. (1990) that terpenes, despite being valuable enhancers for ketoprofen, showed no significant increase in the thermodynamic activity of it.

The predominant factors determining the diffusion of a permeant across the skin are partition coefficient, thermodynamic activity, and diffusion coefficient (Hadgraft, 2001). Results of this study suggest that thermodynamic activity of LHCl with or without terpenes had no significant role in LHCl permeation. Increased enhancements in LHCl transdermal fluxes, thus, may be attributed to an increased partitioning and disruption of the stratum corneum.

Effect of Terpenes on Partition Coefficient

The data from Table 1 manifest that, although all the terpenes used in the permeation study significantly enhanced the partition coefficient of LHCl ($P < 0.05$) in comparison to

TABLE 1
Saturation Solubility and Transdermal Permeation Data of Labetolol Hydrochloride Across Rat Abdominal Skin in Presence and Absence of Terpenes

	Flux, J*	T*	$K_p\ 10^3$*	$D*10^2$	*P**	ER veh*	ER wat*	Solubility* (mg/ml)
Water	2.14 (0.356)	2.71 (0.56)	0.71	0.14 (0.0006)	0.077 (0.02)	—	—	28.87 (3.4)
Vehicle	3.84 (0.627)	2.24 (0.86)	1.28	0.17 (0.0009)	0.115 (0.04)	1.79 (0.25)	—	22.14 (2.2)
Basil oil	178.6 (28.78)	0.66 (0.23)	59.54	0.57 (0.0021)	1.576 (0.32)	83.39 (9.3)**	46.52 (3.8)	23.64 (4.1)
Camphor	161.4 (25.29)	0.48 (0.17)	53.80	0.77 (0.0033)	1.044 (0.29)	75.35 (6.9)	42.03 (4.1)	21.27 (3.5)
Geraniol	115.7 (21.30)	0.83 (0.32)	45.39	0.45 (0.0019)	1.508 (0.41)	63.58 (5.2)	35.47 (3.7)	22.87 (2.7)
Thymol	92.6 (13.24)	0.98 (0.19)	30.87	0.38 (0.0008)	1.214 (0.21)	24.12 (4.2)	43.24 (2.2)	23.54 (4.6)
Clove oil	86.5 (15.11)	1.16 (0.42)	28.83	0.32 (0.0015)	1.344 (0.27)	40.39 (5.9)	22.53 (3.1)	21.42 (3.9)

J = Flux in µg cm^{-2} h^{-1}; T is the lag time in hrs; K_p = Permeability coefficient in cm h^{-1}; D = diffusion coefficient in cm^2 h^{-1}; P = partition coefficient of drug between SC and vehicle (with or without terpenes); ER_{wat} and ER_{veh} are enhancement ratios.

*The results are the mean of triplicate observations. SD values are given in parentheses.

**Statistically significant at $P < 0.05$.

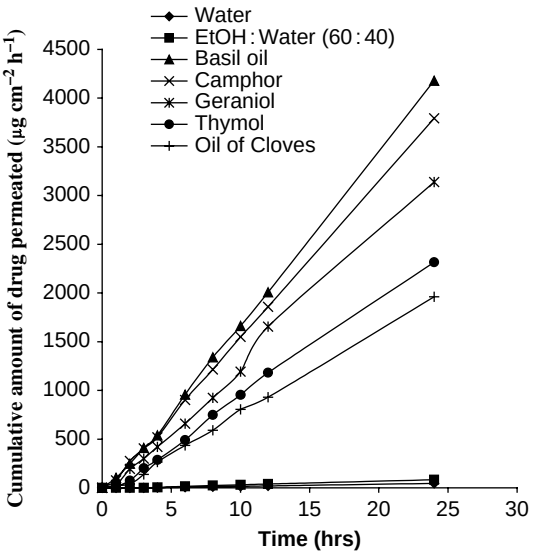

FIGURE 1. Effect of terpenes and essential oils on permeation of labetolol hydrochloride across rat skin.

control (vehicle), there was no significant difference ($P < 0.05$) among the partition coefficients in the presence of terpenes. So, it can be inferred that despite terpenes enhanced the flux values of LHCl by increasing its partitioning, but the reasons for significant differences observed among flux values of LHCl in presence of terpenes cannot be described by the partition coefficient values only.

The most predominant factor deciding the permeation of a molecule amongst all factors is the diffusion coefficient (Sinha & Kaur, 2000). From the results of the studies (Table 1), it can be said that diffusion coefficient is the dominant factor for altering the permeation of LHCl across the skin.

Effect of Terpenes on the Permeation of LHCl

The present study revealed that the presence of terpenes at 5% w/v in vehicles significantly enhanced ($P < 0.05$) steady state flux of LHCl over the control (Table 1). The steady state flux values obtained after terpenes treatment is in the following decreasing order: basil oil > camphor > geraniol > thymol > clove oil. Among the different terpenes evaluated, enhancement of LHCl with basil oil was significantly different ($p < 0.05$) from other terpenes. The overall flux values obtained were due to the synergistic effect of vehicle system and terpenes. In order to delineate the effect of terpenes and the vehicle system, ER_{wat} and ER_{veh} were calculated. ER_{veh} reflects the permeation of LHCl in presence of the combined action of vehicle system and terpenes, whereas, ER_{wat} reflects the permeation dependent only on the presence of terpenes. As evident from Table 1, the enhancement obtained with synergistic application of vehicle and terpenes is comparatively more than that obtained with neat terpenes.

The prime barrier to the transport of water soluble polar molecules is the intercellular lipids present in the stratum corneum (SC), the uppermost layer of the skin. The stratum corneum is composed of corneocytes surrounded by a matrix of lipid-enriched membranes (Williams & Barry, 2004). This matrix between corneocytes is composed of hydrophobic lipids arranged in lamellar sheets. These hydrophobic lipids are composed mainly of ceramides (50%), cholesterol (25%), and free fatty acids (Law et al., 1995). Ceramides play a critical role in the development of overall lipid matrix organization and are responsible for the barrier property of the SC (Chena, Mendelshhna, Rerekb, & Mooreb, 2000). These are tightly packed in lipid layers because of the hydrogen bonding between the amide group I of one ceramide to the amide group I of the other ceramide, resulting in the formation of tight hydrogen bonding network at the head of ceramides. This hydrogen bonding is the reason for the stability, integrity, and barrier property of the lipid layers in the SC (Moore & Rerek, 2000). In the presence of terpenes, there occurs a competitive hydrogen bonding between ceramides and terpenes that results in the loosening of the tight junctions of lipid layers and the creation of new pathways for the molecular permeation. Terpenes with alcoholic groups interact more competitively with the amide groups of the ceramides than the terpenes having carbonyl group, as the oxygen atom of –OH group is more electronegative. This facilitates the permeation of LHCl across the skin as indicated by the highest ER_{veh} in the presence of basil oil, whereas camphor showed a less ER_{veh} than basil oil; camphor molecule contains ketone oxygen atom which is less electronegative than oxygen atom of alcoholic group.

However, ER_{veh} of geraniol, thymol, and clove oil is lesser than camphor despite the presence of more electrophilic alcoholic oxygen atoms in these volatile oils. It is reported in many studies that permeation of a molecule across the skin in the presence of penetration enhancers is significantly modulated by the physicochemical properties of permeant molecules as well as enhancer molecules. Different physicochemical properties such as boiling point, Log P, and molecular weight of enhancers can significantly enhance the permeation of a molecule (El-Kattan, Asbill, & Michniak, 2000; Jain, Thomas, & Panchagnula, 2002; Narishetty & Panchagnula, 2004). In support of this, permeation of zidovudine and imipramine hydrochloride has been reported to increase when terpenes with lower boiling points were employed. To substantiate this further, a linear relationship was reported for 5-fluorouracil permeation versus log P of terpenes (Williams & Barry, 1991). The different terpenes used in the present investigation were significantly different in their boiling points in the following increasing order (see Table 2): Camphor (204ºC) < Basil oil (213ºC) < Geraniol (228ºC) < Thymol (233ºC) < Clove oil (250ºC). Basil oil, having a low boiling point, showed a higher ER followed by Geraniol, thymol, and clove oil, which reflects agreement with the previous reports.

Boiling point is a reflection of degree of weak cohesiveness or self-association of molecules (Martin, Bustamante, & Chun, 1996). In other words, molecules with lower boiling points are less self-associated and, hence, these are more freely available to interact with lipids of stratum corneum. In the case of basil oil and

TABLE 2
Chemical Class, Composition, and Boiling Points of Study Terpenes

Enhancers	Chemical Class	Composition	Boiling Points(°C)
Basil oil	alcohol	Linalool (62%), Methyl chavicol, geraniol, nerol, citronellol, bornylacetate	213
Camphor	ketone	Camphor	204
Geraniol	alcohol	Geraniol	229
Thymol	alcohol	2-Isopropyl-5-methylphenol	233
Clove oil	alcohol	Eugenol (70–95%), aceto-eugenol (2–3%), Caryophyllene	250

Reference: British Pharmaceutical Codex, Merck Index, and Material safety datasheets of terpene supplying chemical company.

camphor, their molecules are less self-associated with each other, suggesting that lesser energy is required for these molecules to interact with and disrupt SC lipids and this is indicated by their higher ER and D_c. On the other hand, molecules of geraniol, thymol, and clove oil, having higher boiling points, interact less with the SC lipids due to a comparatively higher degree of self-association of their molecules which is reflected by their lesser values of ER_{veh} and D_c of LHCl obtained in the presence of these terpenes in order of their boiling points.

Effect of Terpenes on Lag Time

Terpenes are well known to reduce the lag time of penetrant molecules (Williams & Barry, 2004). Lag times of LHCl were significantly decreased ($P < 0.05$) in the presence of terpenes in the following order camphor < basil oil < geraniol < thymol < clove oil < vehicle < water (Table 1). As per the equation for calculating D_c (see data analysis section), lag time is proportionally related to the diffusional path length of a molecule and, in turn, diffusional path length is related to the tortuousity of the intercellular pathway in the SC. Absolute ethanol extracts the lipids and water from SC intercellular space (Inamori, Ghanem, Higuchi, Srinivasan, 1994). This extraction, though, increases porosity, but extraction of water leads to interdigitization of the lipid layer, which leads to the increased tortuousity (Li et al., 1998; Peck, Ghanem, & Higuchi, 1994,). This may be the reason that despite the extraction of lipids from the SC, lag times observed with the neat vehicle and water per se were not significantly different. However, a reduced lag time was observed with the vehicle in the presence of terpenes, which according to Magnusson et al. (1997) may be because of the less and slower extraction of lipids by ethanol and a greater and faster lipid extraction at the same site by terpenes. Vaddi et al. (2002) stated that higher values of activity and diffusion parameters result in a shorter lag time value. As evident from Table 1, values for D_c show agreement with observed lag times.

Effect of Activation Energy on LHCl Permeation

The activation energy of molecules to cross the skin barrier depends on its physiochemical properties and the path of diffusion. Diffusional path in the SC can be altered by changing its lipid layer fluidity with the help of penetration enhancers. Activation energies for LHCl were determined in water, vehicle, and 5% w/v basil oil in vehicle. Arrhenius plots were obtained by plotting log Kp values versus 1/T and were found linear as shown in Figure 2. Linearity of the plots indicates that no significant structural variation or phase transition had occurred in the SC (Narishetty & Panchagnula, 2004). The activation energies for LHCl obtained using the Arrhenius equation were 23.16, 18.71, and 10.98 kcal/mole for water, vehicle, and 5% w/v basil oil in vehicle, respectively. A lowering of Ea in the presence of 5% w/v basil oil reflects alteration in the lipids bilayers of SC. Cornwell and Barry (1993) reported that activation energy for ion transport across human skin is 4.08 kcal/mole. Also, it is well known that ion transport across the skin takes place via aqueous shunt routes in the skin (Cornwell & Barry, 1993). The activation energy for LHCl in water, found to be 23.16 kcal/mole, is much higher than the activation energy for molecules traversing skin via polar pathway. So, LHCl permeation via aqueous shunt pathways may be ruled out. Further substantiating this, it was reported that activation energies for 5-flourouracil, terolodine HCl, and imipramine HCl were 20.6, 20, and 21.24 kcal/mole, respectively (Cornwell & Barry, 1993; Jain & Panchagnula, 2003; Ogiso, Hirota, Iwaki, Hino, & Tanino, 1998,) for diffusion across skin, and it was suggested that these molecules cross skin barrier through the intercellular lipid pathway. As the activation energy of LHCl is similar to those of the above molecules, it can be concluded that the former might be traversing the skin by intercellular lipid pathway.

As discussed previously, lipid layers in SC are held together by a hydrogen bonding network (both lateral and transverse).

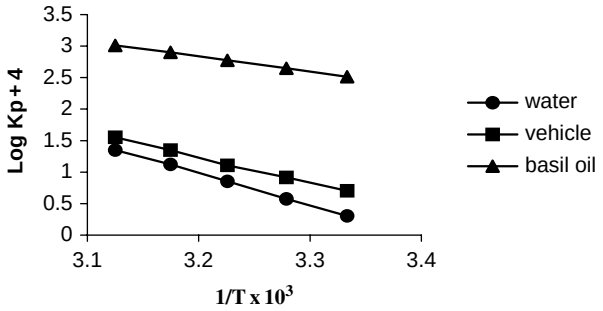

FIGURE 2. Arrhenius plot between log K_p and 1/T for determination of activation energy for basil oil facilitated skin permeation of labetolol hydrochloride.

Higher activation energy is required to push the molecules across the transverse hydrogen bonding holding the lipid lamellae in SC. When the SC is treated with terpenes, a competitive hydrogen bonding between the lipids and terpenes occurs, leading to the disruption of the barrier provided by the transverse hydrogen bonding between lipid bilayers. Disruption of this barrier leads to the lower activation energies of molecules to diffuse across skin. In agreement to the above discussion, lower activation energies for LHCl to permeate across skin were found in the presence of 5% w/v basil oil. Furthermore, the activation energy in presence of basil oil is similar to the activation energy for polar molecule across human skin, hence, it can be concluded that basil oil created new polar pathways in the SC lipids across which LHCl is permeating.

CONCLUSION

Basil oil, a volatile oil containing terpenes and having alcoholic groups, has been found to be an effective penetration enhancer for LHCl, a hydrophillic drug. It is concluded that basil oil enhances the permeation of LHCl by compromising the intercellular lipid barrier of SC. The oil may be potentially used as an enhancer in the formulation of transdermal drug delivery systems of hydrophillic drugs a la LHCl. It would be interesting to study the individual effects of various constituents of basil oil on skin permeation of potential drug candidates.

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