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Original Research Paper

Investigating the potential of essential oils as penetration enhancer for transdermal losartan delivery: Effectiveness and mechanism of action

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ABSTRACT

The effect of tea tree oil (TTO), cumin oil (CO), rose oil (RO) and aloe vera oil (AVO) on the skin permeation of losartan potassium (LP) was investigated. *In vitro* skin permeation studies were carried out using rat skin. The mechanism of skin permeation enhancement of LP by essential oils treatment was evaluated by FTIR, DSC, activation energy measurement and histopathological examination. Both concurrent ethanol/enhancer treatment and neat enhancer pretreatment of rat SC with all the oils produced significance increase in the LP flux over the control. The effectiveness of the oils as the penetration enhancers was found to be in the following descending order: AVO > RO > CO > TTO. However, only AVO was the only enhancer to provide target flux required to deliver the therapeutic transdermal dose of LP. FTIR and DSC spectra of the enhancer treated SC indicated that TTO, CO, RO and AVO increased the LP permeation by extraction of SC lipids. The results of thermodynamic studies and histopathological examination of AVO treated SC suggested additional mechanisms for AVO facilitated permeation i.e. transient reduction in barrier resistance of SC and intracellular transport by dekeratinization of corneocytes which may be attributed to the presence of triglycerides as constituents of AVO. It is feasible to deliver therapeutically effective dose of LP via transdermal route using AVO as penetration enhancer.

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1. Introduction

Hypertension is the most common cardiovascular disease worldwide; hypertension is cited as the leading cause of non-communicable disease mortality worldwide. It is a progressive disorder, which if not effectively managed results in a greatly increased probability of coronary thrombosis, strokes and renal failure. Moreover, it requires long-term treatment that may result in poor patient compliance with conventional dosage forms due to greater frequency of drug administration [1]. 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. Skin is a complex organ that serves to protect the living organisms from chemical, physical, and biological intrusion. In an average adult it covers an area of about 1.73 m^2 and receives one third of circulating blood through the body at any given time. Thus, it presents an excellent opportunity for systemic delivery of active pharmaceutical ingredients [2].

Transdermal delivery of drugs promises many advantages over oral or intravenous administration, though human skin provides an effective barrier to the permeation of most drugs in the form of stratum corneum (SC) [3,4]. Success of the transdermal route depends on the ability of drugs to breach this barrier and permeate the skin at a rate sufficient to attain effective plasma concentration. There are many approaches were employed to enhance the skin permeation rate of active moieties. However, the most convenient and widely implemented approach is the use of chemical penetration enhancers [5,6] such as DMSO, DMF, azone, ionic surfactants, but their use are also associated with unpleasant and toxic side effects. In recent years there has been a search for natural compounds as permeation enhancers to improve drug permeation that also exhibit low toxicity while maintaining their enhancing activity.

The natural absorption promoters documented so far include essential oils, terpenes, terpenoids, fatty acid esters, fatty acid glycols, and herbal extracts. The essential oils are nontoxic, non-allergic, and compatible with drug and excipients have received much attention of researchers and found one of the promising groups of candidates to be employed as clinically acceptable penetration enhancers. Essential oils present a large range of chemically acceptable and relatively safe penetration enhancers to aid percutaneous drug delivery and are considered as GRAS (generally regarded as safe) compounds for medicinal use. They have been reported to use for permeation enhancement of both hydrophilic and lipophilic drugs. They cause no skin toxicity or if any, only mild irritation [7].

Very recently, Patil and Saraogi, reviewed the details of different natural products including essential oils as potential permeation enhancer for transdermal delivery of various actives [8]. Antecedently, the feasibility of natural oils viz. corn (maize) oil, groundnut oil and jojoba oil as penetration enhancer was reported for enhanced transdermal olanzapine delivery [9]. The author claimed that the magnitude of flux enhancement factor with corn oil, groundnut oil and jojoba oil was 7.06, 5.31 and 1.9 respectively at 5 mg/ml concentration in solvent system. Amongst the oil used, corn oil containing unsaturated fatty acids was found to be promising natural permeation enhancer for transdermal delivery of olanzapine with greatest cumulative amount of drug permeated (1010.68 $\mu g/cm^2/h$) up to 24 h and caused no skin irritation. In this quest, the present work was carried out to monitor the effect of commonly used essential oils namely, tea tree oil (TTO), cumin oil (CO), rose oil (RO) and aloe vera oil (AVO) on skin permeation of losartan potassium (LP) and to elucidate the mechanism of skin permeation enhancement.

The interactions of penetration enhancers with SC lipids and proteins (mechanism of permeation) can be elucidated with instrumental methods such as Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). FTIR provides the information about the molecular and conformational changes of lipids and proteins, whereas the DSC provides the information about their thermotropic behavior [10]. FTIR and DSC techniques were used to investigate the mechanism of LP permeation in presence of test enhancers.

Briefly, LP (Biopharmaceutics classification system class 3 drug) is an orally active angiotensin II receptor antagonist indicated for the treatment of hypertension. The normal daily dose of LP is 50 mg and in case of severity 100 mg dose is given. The drug possesses extensive hepatic first pass metabolism (67%) and short biological half-life (2 h) [11,12]. Therefore, LP bypass hepatic first pass metabolism, hence its dose can be reduced in transdermal formulation significantly. All these properties, such as extensive hepatic first pass metabolism (67%), short biological half-life (2 h), low dose (25–50 mg), low oral bioavailability (33%), log P = 4.5, and low molecular weight (461.01 Da), make it suitable for transdermal drug delivery system [13–16]. In the present study, TTO, CO, and RO were selected as essential oils and these were compared with AVO as penetration enhancers to promote the percutaneous absorption of LP.

2. Materials and methods

2.1. Materials

LP was received as gratis sample from Ranbaxy Research Laboratories Ltd, Gurgaon, India. All the investigated oils namely tea tree oil (TTO), cumin oil (CO), rose oil (RO) and aloe vera oil (AVO) were purchased from authentic source (Sigma–Aldrich Chemicals Private Limited New Delhi, India). Sodium chloride, Sodium Hydroxide, ethanol, Potassium sulfate, Sodium azide and Sodium bromide were purchased from S.D. Fine chemicals, India. Potassium dihydrogen orthophosphate were purchased from Merck India Ltd. India. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Spectrochem Pvt Ltd, Mumbai, India. All other chemicals used were of reagent grade. All materials were used as received. Double distilled water was used for all experiments.

2.2. Animals

Wistar rats (200–250 g) were supplied by Central Animal House of Hamdard University and inhabited under standard

laboratory conditions in 12 h light/dark cycle at 25 ± 2 °C. Animals were nourished with pellet diet (Lipton, India) and water *ad libitum*. The animals were received after the study was duly approved by the University Animal Ethics Committee, and CPSCEA (Committee for the purpose of control and supervision on experiments on animals), Government of India.

2.3. *Preparation of rat skin and* in vitro *skin permeation studies*

Wistar rats were sacrificed with prolonged ether anesthesia; the abdominal skin of each rats were excised. Hairs on the skin of animal were removed with electrical clipper; subcutaneous tissues were surgically removed, and dermis side was wiped with isopropyl alcohol to remove residual adhering fat [17,18]. The skin was washed with phosphate buffer saline (PBS; pH 7.4), wrapped in aluminum foil and stored in a deep freezer at −20 °C until further use (used within two weeks of preparation). On the day of experiment, skin was brought to room temperature and skin samples were mounted over the diffusion cells in such a way that SC side faced the donor compartment whereas the dermis faced the receiver compartment [4,19]. The effective diffusion area between two half cells was 4.89 cm^2 and the capacity of each half cell was 10 ml. The receiver fluid was equilibrated at 37 ± 0.5 °C and stirred with magnetic stirrer at a speed of 600 rpm [20,21]. The *in vitro* permeation study was done in two following ways.

2.3.1. *Study 1: donor solution containing penetration enhancer in the vehicle*

In study 1, saturated solution of LP in ethanol: PBS (1:9) mixture with test penetration enhancer was added to the donor compartment.

2.3.2. *Study 2: skin pretreated with penetration enhancers*

While in study 2, the skin was pretreated with test penetration enhancer for 2 h [22]. The oil was removed, followed by washing the skin with distilled water blotting with a tissue paper and drying at room temperature for 15–30 min. To the donor compartment, saturated solution of LP in distilled water was added.

To both the donor and receptor solutions, sodium azide (0.0025%, w/v) was added to prevent any microbial growth. The samples (2 ml) were periodically withdrawn from the receptor compartment up to 24 h with replacement of fresh PBS to the receiver phase. The samples were filtered and analyzed for the drug content by HPLC method [23]. The HPLC system consisted of a series LL-10AT VP pump (Shimadzu, Japan) equipped with a UV detector. Separation was achieved using RP-18 column (250 mm × 4.6 mm I.D.). The isocratic mobile phase consisted of phosphate buffer (pH 3): acetonitrile (60:40 v/v) was used. The flow rate was set to 1 ml/min. UV-HPLC detection was performed at 273 nm.

2.4. *Permeation enhancement mechanistic studies*

2.4.1. *FTIR study*

On the day of experiment, skin was brought to room temperature and was treated with 1 M sodium bromide solution in distilled water for 4 h. The epidermis from full thickness skin was separated using cotton swab moistened with water. Epidermal sheet was cleaned by washing with distilled water and dried under vacuum and examined for cuts or holes if any. SC samples were prepared by floating freshly prepared epidermis membrane on 0.1% trypsin solution for 12 h. Then, SC sheets were cleaned by washing with distilled water. The SC specimen was cut into pieces of 1 cm^2 and soaked in screw-capped vials with 1 ml of neat test enhancer for 2 h. After treatment with oil, the epidermal piece was blotted with tissue paper and dried at room temperature for 15–30 min. FTIR spectra were recorded on FTIR spectrophotometer in the region of 4000–400 cm^{-1}. The spectrum was recorded before and after the enhancer's treatment.

2.4.2. *DSC study*

For DSC studies, 5 mg SC samples were sealed in aluminum hermetic pans placed in instrument. The SC samples (with or without enhancer treatment) were scanned on a DSC6 (Perkin Elmer, Germany). Scanning was done at the rate of 10 °C/min over the temperature range 10 °C–200 °C [24].

2.4.3. *Determination of activation energy*

In vitro permeation studies of LP across rat skin were carried out at various temperatures (27 °C, 37 °C, and 47 °C of receptor medium) after pretreatment with AVO for 2 h. Permeability coefficients (k_p) were calculated at each temperature and subsequently log k_p values were plotted against 1/T. Activation energies of LP were then calculated from Arrhenius relationship [25,26].

$$\log K_p = E_{act}/2.303\,RT + \log p^0$$

where E_{act} is the activation energy, R is gas constant (1.987 K cal/mol), and T is the temperature in K, p^0 is the Arrhenius factor.

2.4.4. *Histopathological studies*

Histopathological studies were carried out for elucidation of mechanism of penetration enhancement and skin irritation potential of the investigated enhancer. In this study, the skin hairs of an albino rat was removed using electrical clipper and skin was treated with AVO for 24 h. After 24 h, rat was sacrificed and the skin samples from treated and untreated (control) area were taken. Each specimen was stored in 10% formalin solution in PBS. The specimen was cut into sections vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing, and stained with hematoxylin and eosin. These samples were then observed under light microscope (Motic, Japan) and compared with control sample. In each skin sample, three different sites were scanned and evaluated for elucidation of mechanism of penetration enhancement [3,4,27].

2.5. *Data analysis*

Flux is the rate of change of the cumulative amount of drug passes per unit area and time through the skin. The flux was determined as the slope of steady state portion of the curve between the cumulative amounts of drug permeated (μg/cm^2)

Table 1 – Skin permeation parameters of LP using rat skin treated with ethanol: PBS mixture containing investigated penetration enhancer (Study 1) and pretreated rat skin with the penetration enhancers (Study 2).

Penetration enhancer	Flux (μg/cm²/h)		Pb × 10^{-5} (cm/h)		Enhancement ratio		% increase in flux		Lag time (h)	
	Study 1[a]	Study 2[b]	Study 1[a]	Study 2[b]	Study 1[a]	Study 2[b]	Study 1[a]	Study 2[b]	Study 1[a]	Study 2[b]
Control[c]	255 ± 1.7[d]	220 ± 1.7[d]	41.57	35.86	1	1	–	–	2.22 ± 0.5	2.26 ± 0.6
TTO	261 ± 1.1[e]	226 ± 1.7[e]	42.38	36.84	1.02	1.05	2.35	2.72	0.62 ± 0.10	0.66 ± 0.11
CO	287 ± 1.2[f]	283 ± 5.7[f]	46.78	46.13	1.13	1.31	12.54	28.64	0.32 ± 0.03	0.30 ± 0.02
RO	343 ± 1.7[f]	372 ± 1.1[f]	55.91	60.64	1.34	1.73	34.51	69.09	0.12 ± 0.01	0.08 ± 0.01
AVO	349 ± 2.3[f]	510 ± 2.8[f]	56.89	83.13	1.36	2.36	36.86	131.8	0.05 ± 0.005	0.005 ± 0.002

[e]Non-significant ($P > 0.05$).
[f]Significant ($P < 0.05$).
[a] Study 1: Donor solution containing ethanol: PBS (1:9) and penetration enhancer.
[b] Study 2: SC pretreated with the penetration enhancers for 2 h.
[c] Respective controls were subjected to same treatment except penetration enhancer.
[d] Mean ± SEM values of three observations.

and time in (h). The permeability coefficient (k_p) was calculated by dividing the flux by the initial drug concentration per unit area of the skin (μg/cm²). The enhancement ratio (ER) was calculated by dividing k_p in presence of enhancer by k_p without enhancer (control).

Therapeutic transdermal daily dose (T_d) of LP was calculated by the following equation [24].

$$T_d = D_o \times F/100$$

where, F is the bioavailability in percentage after oral administration (33% for LP) and D_o is the oral dose (25 mg for LP).

The permeated daily dose (D_{ss}) was calculated from the following equation.

$$D_{ss} = J \times T_a \times t$$

where, J is the LP flux in μg/cm²/h, T_a is the area of diffusion and t is the duration of treatment.

2.6. Statistics analysis

The data was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad Prism version 3.00 for Windows (San Diego, CA, USA). Statistical significance will be assumed when $P \leq 0.05$.

3. Results and discussion

3.1. In vitro skin permeation studies

The results of *in vitro* skin permeation studies revealed that the flux of LP from saturated aqueous solution without enhancer (control) was 220–255 μg/cm²/h (Table 1). This rate of skin permeation would enable 6.45 mg of LP absorbed in 24 h which is well short of daily transdermal therapeutic dose of LP (8.25 mg). In order to enhance the transdermal flux of LP across the rat skin various essential oils namely TTO, CO, RO were evaluated for their permeation enhancement potential.

Two different sets of skin permeation studies were carried out. In study 1 (Fig. 1), 5% v/v of test enhancer was added to the saturated solution of LP in the vehicle i.e. ethanol: PBS (1:9) and the mixture was placed in the donor cell. In study 2 (Fig. 2), SC was pretreated with test penetration enhancer and then exposed to saturated solution of LP in the vehicle kept in the donor cell. The two studies were conducted to compare the effectiveness of the penetration enhancer pretreatment and concurrent vehicle-enhancer treatment of SC.

In study 1 (Table 1), all the essential oils-vehicle treatments produced significant differences ($P < 0.05$) in the LP flux over the control with the exception of TTO ($P > 0.05$). Individual comparison between the enhancer-vehicle treatments revealed highly significant difference ($P < 0.001$) in case of TTO-ethanol v_s CO-ethanol, CO-ethanol v_s RO-ethanol and RO-ethanol v_s TTO-ethanol.

The results of the study 2 (enhancer pretreatment) indicated similar pattern of permeation enhancement. Highly significant increment in LP flux ($P < 0.001$) was obtained by the essential oils pretreatment over the control except TTO ($P > 0.05$). On individual comparison between various pretreatments, highly significant difference ($P < 0.001$) was observed between TTO v_s CO., CO v_s RO and RO v_s TTO (Table 1).

Inter study comparison presented a significant difference ($P < 0.05$) between the LP flux obtained on concurrent vehicle-

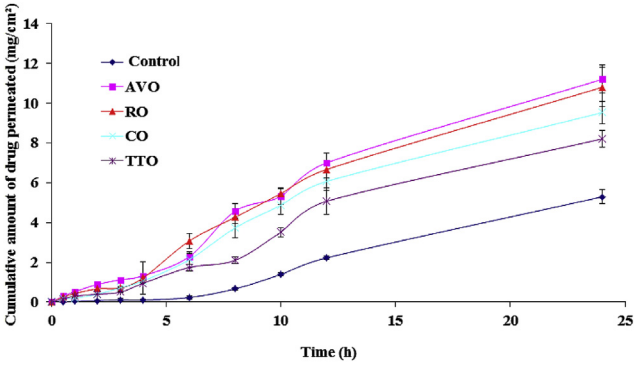

Fig. 1 – Skin permeation profile of LP on concurrent treatment of test penetration enhancers with saturated solution of LP in the vehicle i.e. ethanol: PBS (1:9) (Study 1).

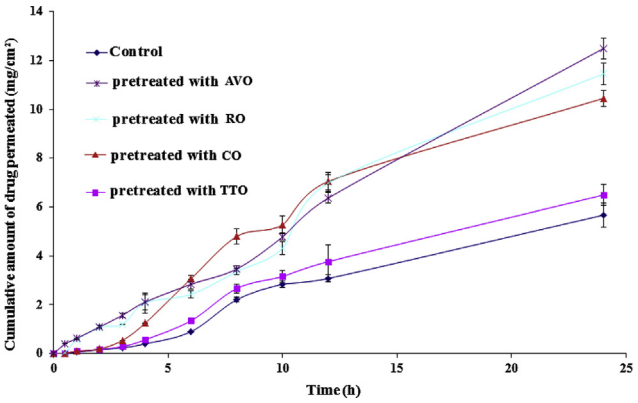

Fig. 2 – Skin permeation results of LP on pretreatment with penetration enhancers followed by treatment with saturated solution of LP in the vehicle i.e. ethanol: PBS (1:9) (Study 2).

enhancer treatment (study 1) and the enhancer pretreatment (study 2) for all the oils except in case of CO (Table 1).

The results of the above two studies suggested the effectiveness of investigated essential oils as penetration enhancer in the following order: RO > CO > TTO. As RO contains oxygen containing hydrophilic terpenes (citronellol, geraniol, nerol, farnesol, geranic acid and eugenol), it has emerged as the most effective absorption promoter amongst the test essential oils for LP, a hydrophilic drug. The results are in conformity with previous reports [28–30]. Although RO was found to be most effective penetration enhancer for LP and it significantly enhanced the LP flux over the control, however it failed to produce the target flux required to deliver the required therapeutic dose for 24 h.

In the light of above observations, literature was reviewed for other probable penetration enhancer to achieve the therapeutic dose of LP delivered by the transdermal route. Our search led to US patent: 6746689, by Wilfried et al. [31], which claimed the use of aloe vera as the topical penetration enhancer for delivering the required amount of a local anesthetic intradermally. However there are no reports on the use of aloe vera products as the systemic penetration enhancers for transdermal use. Hence, we investigated the feasibility of therapeutically effective transdermal delivery of LP using AVO as the penetration enhancer.

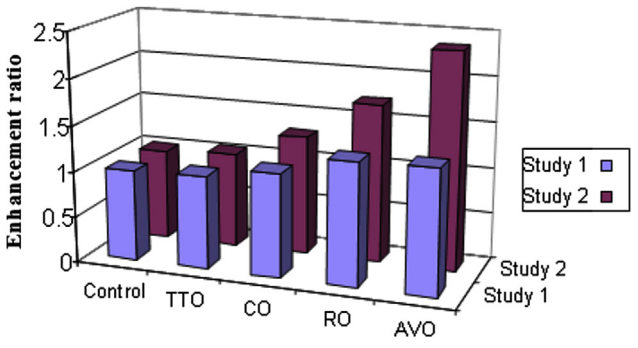

Fig. 3 – Comparison of enhancement ratio between Study 1 and Study 2.

Our results demonstrate that there was 1.36 fold and 2.36 fold enhancement in LP flux by AVO over the control in study 1 and study 2, respectively (Fig. 3). Thus, AVO was found to be the more effective penetration enhancer for LP than all the test essential oils. The AVO pretreatment of SC produced a target flux of 510 μg/cm^2/h which would allow required therapeutic dose of LP (8.25 mg) delivered transdermally in 24 h.

3.2. *Permeation enhancement mechanistic studies*

3.2.1. *FTIR study*

The FTIR spectrum of untreated SC shows various peaks due to molecular vibrations of lipids and proteins in the SC. The absorption bands from 3000 to 2700 cm^{-1} are due to the C–H stretching motions of the alkyl groups present in both proteins and lipids. The signal linked to proteins is a broad band, rather weak compared to the lipids absorption which exhibits four peaks at around 2850 cm^{-1}, 2920 cm^{-1}, 2870 cm^{-1}, and 2955 cm^{-1} [29]. The bands at 2920 and 2850 cm^{-1} are due to the asymmetric CH_2 and symmetric CH_2 vibrations of long chain hydrocarbons of lipids, peaks at 2955 cm^{-1} and 2870 cm^{-1} are due to the asymmetric and symmetric CH_3 vibrations respectively [32]. These narrow bands are attributable to the long alkyl chains of ceramides, cholesterol and fatty acids, which are the major components of the SC lipids. FTIR spectra of SC treated with each oil decreased the height and area of CH_2 stretching, indicating that the lipids were extracted from SC by all the penetration enhancers (Fig. 4) though the lipid extraction was higher with RO and AVO.

The two strong bands at 1650 and 1550 cm^{-1} are due to the amide I and amide II stretching vibrations of SC proteins. The amide I bands arises from C=O stretching vibration and the amide II bands from C–N bending vibration. The amide I band, consisting of component bands, represents various secondary structures of keratin.

The change in amide I stretching peak on treatment with penetration enhancers is due to the extraction of proteins (Fig. 5). The frequencies of amide bands especially amide I are sensitive to shift to higher or lower frequencies according to the change in protein conformation [24]. The amide I band

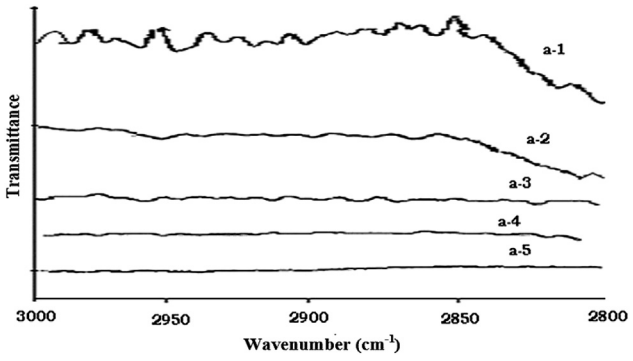

Fig. 4 – FTIR spectra of rat skin after treatment with penetration enhancers. Changes in lipid C–H stretching (2920 cm^{-1}) vibrations; the spectra are arranged from top to bottom: a-1; control, a-2; TTO. Pretreated SC, a-3; CO. pretreated SC, a-4; RO pretreated SC, a-5; AVO pretreated SC.

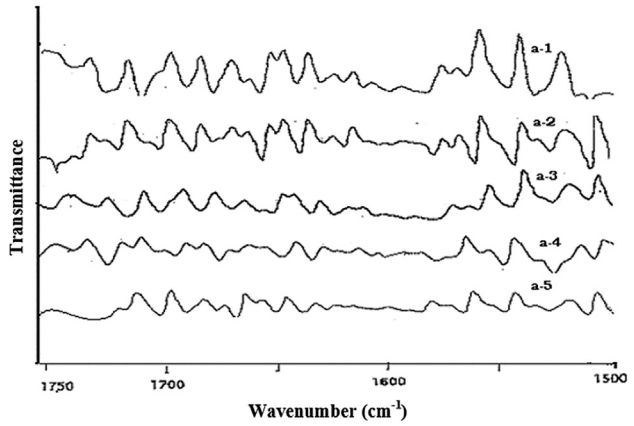

Fig. 5 – FTIR spectra of rat skin after treatment with penetration enhancers. Changes in amide I (1640 cm^{-1}) and amide II (1550 cm^{-1}) vibrations. The spectra are arranged from top to bottom: a-1; control, a-2; TTO pretreated SC, a-3; CO pretreated SC, a-4; RO pretreated SC, a-5; AVO pretreated SC.

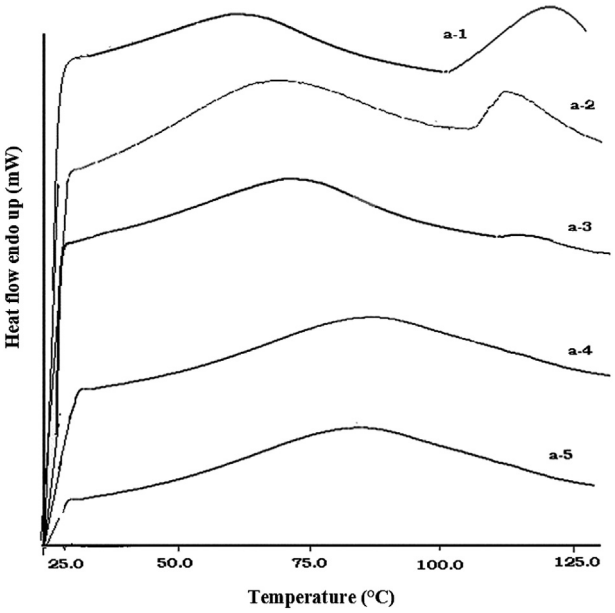

Fig. 6 – DSC thermogram of rat skin showing from top to bottom, a-1; control, a-2; TTO pretreated SC, a-3; CO pretreated SC, a-4; RO pretreated SC, a-5; AVO pretreated SC.

consisting of component bands represents various secondary structures of keratin. As the frequency of the amide I band shifted to higher wave number in comparison to control (untreated SC) (Fig. 5). It is proposed that the essential oils and the AVO used in the present study enhanced the permeation of LP by extraction of SC lipids as well as changes in the protein conformations.

3.2.2. *DSC study*

To further substantiate the mechanism of permeation enhancement, DSC studies were performed. DSC thermogram of untreated SC (control) is shown in (Fig. 6). The first transition T_1 shows up at around 30 °C. The second and third endotherm T_2 & T_3 appear at 62 °C and 101 °C. The first transition T_1 (having the lowest enthalpy) has been attributed to sebaceous secretion or surface contaminations and to minor structural rearrangement within the lipid bilayer [33].

Previous studies have proposed that these transitions are not related to lattice packing variations and assigned it to solid to liquid phase changes for a discrete subset of SC lipids. The second endotherm T_2 is attributed to melting of lipids bilayers [34]. Another lipid peak has been reported in previous studies at 79 °C and 83 °C [34]. But it was not observed in our experiment. The origin of T_3 has been debatable, although recent reports suggest that lipids in the SC are heterogeneously arranged and T_3 might reflect another lipid component. The fourth endotherm which appears at around 100 °C (which was T_3 in our case) has been attributed to intracellular keratin denaturation and is usually heat irreversible [35].

T_1 endotherm is usually ignored because it might not be conclusive in assigning the possible mechanism of skin permeation as it reflects the already compromised state of SC due to solid to liquid phase changes which occur as a natural physiology of the skin. There were definite changes in T_2 and T_3 peaks indicative of extraction of lipid bilayers on each enhancer treatment as compared to control SC (Fig. 6) except TTO which produced only minor changes in T_3 peak. T_3 endotherm disappeared completely in case of AVO which might be assigned to keratin extraction in corneocytes. This suggests the possible intracellular permeation route for LP through corneocytes. The DSC analysis confirms that all the penetration enhancers investigated in the present study enhance the permeation of LP by extraction of SC lipids. AVO also enhances the LP permeation arguably by dekeratinization of corneocytes.

All the enhancers used in our study essentially contain terpenes/triterpenoids. The above results corroborate the findings of previous studies that terpenes enhance the skin permeation of the active pharmaceutical ingredients by extraction of the lipid bilayers [36,37]. Furthermore the intracellular permeation of LP in case of AVO might be due to the presence of other constituents like triglycerides.

3.2.3. *Determination of activation energy*

To further substantiate the mechanism of permeation enhancement of AVO, thermodynamic studies were performed. The Arrhenius plot between log k_p values υ_s 1/T was found to be linear in the temperature range selected (Fig. 7), indicating that no significant structural perturbation or phase transition had occurred within skin membrane [21]. The E_{act} values of LP permeated across rat skin calculated as the slope of Arrhenius plot (Fig. 7) were found to be 17.29 ± 3.5 and 1.83 ± 0.21 K cal mol^{-1} in presence of water without and with AVO pretreatment respectively. The significant decrease ($P < 0.05$) in E_{act} for LP transport across rat skin by AVO indicates that it alters lipid bilayer fluidity.

The high E_{act} values required to traverse the lipid bilayer arise from energy needed to pull molecules free of hydrogen bonds in aqueous vehicle and this creates sufficient free volume within viscous bilayer interiors and pull drug molecules

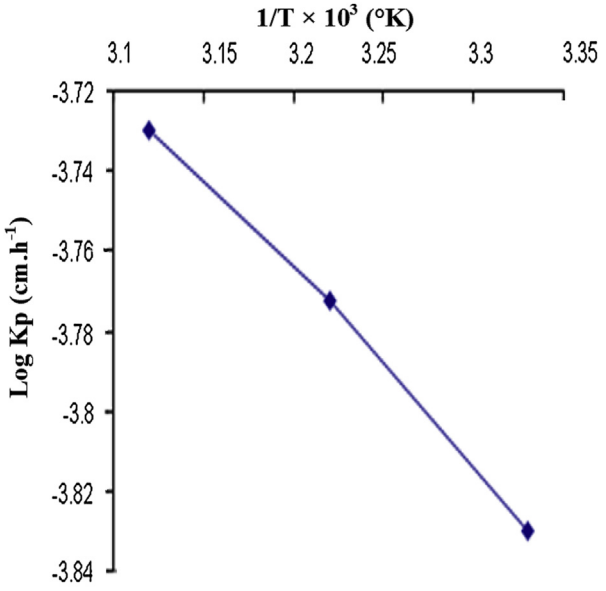

Fig. 7 – Arrhenius plot of LP permeation across rat skin at different temperatures.

free off hydrogen bonds near polar head group regions of lipid bilayer [38]. Alteration of any of these processes may lead to production of E_{act} for transdermal permeation. Accordingly, reduction of E_{act} of LP permeation by AVO may be assigned to the disruption of hydrogen bonding of drug molecule with SC lipids or by creating pre volume in the lipid alkyl chain. Moreover, very low E_{act} of LP transport (1.83 K cal/mol) in presence of AVO suggests that AVO creates new polar pathways by interacting at polar head group region of SC lipids bilayer.

3.2.4. *Histopathological studies*

Fig. 8 shows the photomicrograph of the control (untreated) skin with the well defined epidermal and dermal layers. SC is presenting a layer of distinct keratinized cells (corneocytes). Skin appendages (hair follicles) are clearly visible. On treating the rat skin with the AVO, distinct changes in the keratinized

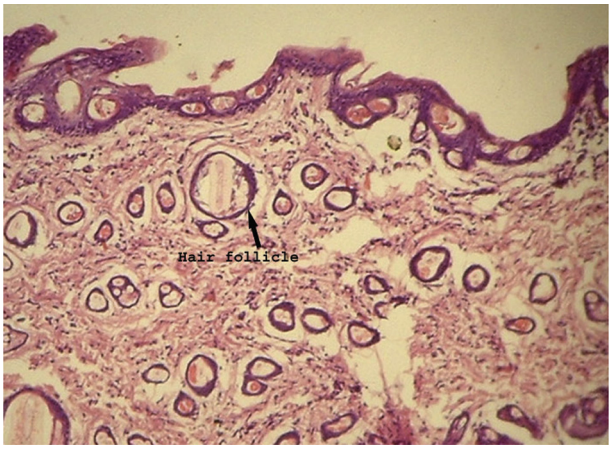

Fig. 8 – Photomicrograph of control skin section (not treated with enhancer) showing normal skin structure. Epidermal and dermal layers are well defined.

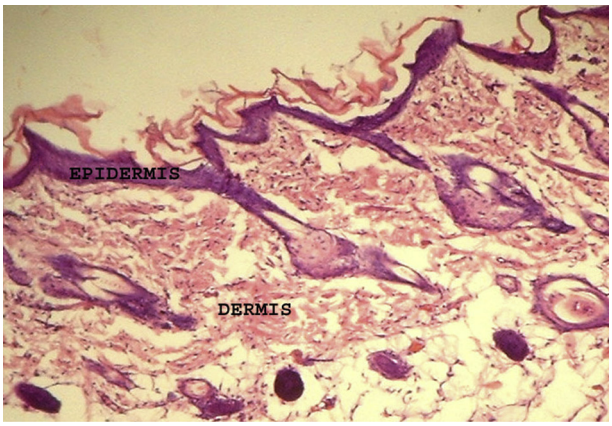

Fig. 9 – Photomicrograph of test skin section (enhancer treated) showing generally normal skin structure except for absent keratin layer.

layer were observed (Fig. 9). Corneocytes were disrupted losing the keratin and proteins. There was no effect on the keratinized cells present in the follicles and the layers of viable epidermis. Dermis and skin appendages were found to be intact. The above observations clearly demonstrate the possibility of transcellular skin transport of LP by AVO evidenced by dekeratinization of corneocytes present in the SC (Fig. 9). There were no signs of skin irritation (e.g. edema, erythema and inflammation) when the rat skin treated with the AVO and the changes observed in the topmost layer (SC) could be attributed to the action of the penetration enhancer (AVO) only.

4. Conclusion

Findings of the above study demonstrate that it is possible to achieve enhanced LP flux by test essential oils (RO, CO, TTO) and AVO. However, only AVO pretreatment provided the target flux required to deliver therapeutic dose of LP percutaneously. FTIR and DSC analysis confirm that all investigated penetration enhancers enhance the permeation of LP by extraction of lipid bilayers in SC. It is concluded that it is feasible to deliver therapeutically effective dose of LP via transdermal route using AVO as penetration enhancer.

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