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The Effect of Herbal Penetration Enhancers on the Skin Permeability of Mefenamic Acid Through Rat Skin

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Abstract:
Introduction: Mefenamic acid (MA) is a strong nonsteroidal anti-inflammatory drug (NSAID), but because of its limited oral bioavailability and the side effects that come with taking it systemically, it is better to apply it topically. The major goal of this study was to see how certain permeation enhancers affected mefenamic acid's in vitro skin permeability. In manufactured Franz diffusion cells, mefenamic acid permeability tests using rat skin pretreatment with several permeation enhancers such as corn oil, olive oil, clove oil, eucalyptus oil, and menthol were conducted and compared to hydrate rat skin as a control.
Methods: The steady-state flux (Jss), permeability coefficient (Kp), and diffusion coefficient are among the permeability metrics studied. The permeability enhancement mechanisms of the penetration enhancer were investigated using Fourier transform infrared spectroscopy (FTIR) to compare changes in peak position and intensities of asymmetric (Asy) and symmetric (Sym) C-H stretching, C=O stretching, C=O stretching (Amide I), and C-N stretching of keratin (Amide II) absorbance, as well as differential scanning calorimetry (DSC) to compare mean transition temperature (Tm) and their enthalpies (H).
Results: Clove oil, olive oil, and eucalyptus oil were the most effective enhancers, increasing flux by 7.91, 3.32, and 2.6 times, as well as diffusion coefficient by 3.25, 1.34, and 1.25, respectively, when compared to moist skin. FTIR and DSC data show that permeation enhancers caused lipid fluidization, extraction, disruption of lipid structures in the SC layer of skin, and long-term dehydration of proteins in this area of the skin.
DISCUSSION AND CONCLUSION: According to the findings, the permeation enhancers utilized improved drug permeability through excised rat skin. The most plausible mechanisms for greater ERflux, ERD, and ERP ratios were lipid fluidization, disruption of lipid structure, and intracellular keratin irreversible denaturation in the SCby eucalyptus oil, menthol, corn oil, olive oil, and clove oil.

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Key words: Mefenemic acid, percutaneous absorption, natural enhancers, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR)

Introduction

Transdermal delivery is based on medication permeation via skin. Transdermal drug delivery has a number of benefits, including controlled and continuous drug delivery, which is important for drugs with short biological half-lives and low therapeutic indices; first-pass intestinal and hepatic bypass; avoidance of gastrointestinal irritation, which is common with oral medications; and easier drug localization at the target site.[1]

Partitioning and diffusion across the SC and viable epidermis, transit into the dermis, and eventually systemic absorption or penetration into deeper tissues are the two key phases in skin permeation. The stratum corneum (SC), the skin's outermost layer, is the most effective barrier against drug penetration. Many techniques have been employed to increase medication access into the lower skin layer and deeper tissues. Permeation enhancers, both chemical and physical, have been developed to help carry high medication concentrations over the skin and into the systemic circulation or deeper tissues. The types of enhancers employed and their mechanisms of action differ.[2] Penetration enhancers work by increasing drug diffusion in the skin, lipid fluidization in the SC, and increasing drug thermodynamic activity in the skin and vehicles, as well as having an influence on drug partition coefficient.

Mefenamic acid, an enolic acid-class nonsteroidal anti-inflammatory medication (NSAID), is often used to treat mild to moderate pain, such as headaches, tooth discomfort, dysmenorrhea, rheumatoid arthritis, osteoarthritis, and other joint problems. Mefenamic acid is classified as a class II biopharmaceutical, meaning it is highly permeable across biological membranes but has poor water solubility.[3] Although oral administration of mefenamic acid is widely used, it necessitates frequent dosing every 6 hours to maintain steady-state plasma concentrations.[4] This route is associated with gastrointestinal side effects such as ulceration, bleeding, or perforation of the stomach, small intestine, or large intestine, which can be fatal; as a result, it is contraindicated in patients with active ulceration or chronic inflammation of the upper or lower gastrointestinal tract.[5] As a result, the only way to get mefenamic acid through the skin is through transdermal administration.

Investigation of the microstructure of intercellular or lipids in the SC layer of skin is necessary to create transdermal medication delivery methods. Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR) have been used in recent research to investigate the organization of lipids and skin microstructure. FTIR analysis of skin may be a useful method for researching the interaction of chemical enhancers with SC that produce bands with varied wave numbers.[6]

Molecular analyses of the entire rat skin were conducted using DSC and FTIR to determine the mechanism by which the characteristics of enhancers/retardants vary in a specific medium.[7] Several infrared spectral bands of the skin are attributed to vibration of protein and lipid molecules in the SC.[8] Lipid vibration is a good way to look at the microstructure of lamellar lipids in the intercellular region of the SC layer. Many of the skin's infrared spectral bands are caused by the vibration of protein and lipid molecules in the SC. Lipid vibration is a good predictor of the architecture of lamellar lipids in the intercellular area of the SC layer. stratum

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corneum stretching vibrations of C-H symmetric vibration (about 2850 cm^{-1}) and C-H asymmetric vibration (around 2920 cm^{-1}) have been recorded. The wave number and width of C-H stretching peaks increase when the lipids in the SC fluidize. If the shift is to a higher wavenumber (blue shift), it means that the SC membrane (lipid bilayer) is fluidizing, which contributes to the breakdown of the barrier properties, allowing more material to pass through the SC. Lipid groups, on the other hand, reorient, producing a change in wave number (e.g., red shift) and strengthening of subcutaneous barrier characteristics, which slows permeant transit through the skin. The phase transition of the lipids is illustrated by an increase or drop in the band position (wavenumber) of the signals at 2920, 2850, and about 1738 cm^{-1} when the penetration modifier acts on the lipid pathway.[9-12]Thermal analysis methods such as differential scanning calorimetry (DSC) have been utilized to investigate thermal transitions in mammalian stratum corneum. Thermodynamic analysis techniques like differential scanning calorimetry (DSC) have been used to study temperature transitions in the stratum corneum. The skin's barrier function is controlled by the SC, which is the epidermis' outermost layer.[13]
The DSC method is commonly used to study lipid melting, lipid bilayer phase transitions, and protein denaturation in the SC layer. A DSC investigation was planned to learn more about the lipid components and protein conformational stability of the entire skin rat that had been treated with enhancers.[14]
The thermotropic behavior of treated skin was examined by comparing mean transition temperatures (Tm) and enthalpies (H). Any decrease in Tm might be the result of lipid breakdown in the bilayer and irreversible protein denaturation in the SC. Enthalpy loss is often linked to lipid fluidization in lipid bilayers and protein–lipid interactions.[15]

MATERIALS AND METHODS

The ramopharmin pharmaceutical firm donated mefenamic acid (Tehran, Iran). Barij Essence Iranian Company in Kashan (Iran) provided eucalyptus oil, olive oil, corn oil, clove oil, and menthol.

Animal experiments

For the in vitro permeation investigation, male Wistar rats weighing 200–250 g were employed. The abdomen skin hair was meticulously cut using an electric clipper and razor after the animal was sacrificed under ether anesthesia. The skin was dissected, and any excess subcutaneous fat from the dermal surface was removed. The animals were cared for accordance to the guidelines for the care and use of laboratory animals, and the experiments were approved by the Ahvaz Jundishapur University of Medical Sciences' Ethical Committee (IR.AJUMS.REC.1396.295). The National Academy of Sciences issued recommendations, which were published by the National Institutes of Health (U.S. Department of Health and Human Services, Office of Laboratory Animal Welfare).[16,17]

Skin permeation experiments

Permeation tests were conducted using specifically built diffusion cells with an effective area of about 4.906 cm^2. In the donor phase, 2 ml of each natural permeation enhancer was applied to the surface of the skin for 2 or 4 hours. After that, the donor and receptor compartments were rinsed and filled with 5ml of 1% w/v mefenamic acid suspension and 30 mL of phosphate buffer solution (pH 7.4), respectively. As a control, we utilized fully hydrated samples. On a magnetic

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stirrer with a heater, the diffusion cell was inserted and clamped in a water bath at 37± 0.05 °C. A tiny magnetic bead was used to agitate the receptor medium at 200 rpm. At predefined time intervals (0.5, 1, 2, 3, 4, 5,6,7,8, and 24 h), 2 ml of the receptor medium was removed and replaced with an equivalent amount of fresh buffer. The quantity of mefenamic acid was assessed using a UV spectroscopic technique at 289 nm after the samples were filtered.[18, 19]

Data Analysis

The total quantity of mefenamic acid that penetrated into the receptor via each unit area of the diffusion surface was determined and displayed as a function of time. The linear component of the permeation curve's slope was used to compute steady state flux (mg/cm2.h). Permeability coefficient (K_p, cm/h) of mefenamic acid through the skin was calculated using Equation 1:

$K_p = \frac{J_{ss}}{C_v}$ …………….…………….. (Equation 1)

Where J_{ss} and C_v are steady state flux and initial concentration of mefenamic acid in receptor compartment, respectively. Also, lag time (Tlag) and apparent diffusivity coefficient (Dapp) parameters were calculated. As h does not represent the actual length of the pathway, the D calculated from this formula is also apparent to D. The value of Dapp is calculated from Equation2:

D app= $h^2/6T_{lah}$…………….…………….. (Equation 2)

Enhancement ratios (ER) were calculated from equation 3: [21]

$ER = \frac{\text{permeability parameter after treatment}}{\text{permeability parameter before treatment}}$ …………….…………….. (Equation 3)

Statistical comparison was made using one-way ANOVA and $p<0.05$ was considered statistically significant.

Lag time (T_{lag}) of drug obtained from the skin along the line of equilibrium to the axis of time in the cumulative curve of the drug. The value of D is calculated from Equation 2: $D = h^2/6Tlag$. As h does not represent the actual length of the pathway, the D calculated from this formula is also apparent to D. Seeing all calculations are based on the steady-state region, the cumulative flow rate of the drug is determined, so the establishment of sink conditions is indispensable for the citation of these parameters. In this work, the maximum concentration established in the receptor phase was less than 10% of the saturation solubility of the drug in the receptor phase, and therefore, a steady concentration gradient was established during the experiments, and with these conditions, a steady state flux was computed.

Differential scanning calorimeter (DSC)

Using a DSC (Mettler-Toledo DSC[1] System) equipped, the changes in the structure of the entire skin caused by permeation enhancers were investigated. The skin samples were submerged in each natural permeation enhancer for 4 hours before being blotted clean. In hermetically sealed aluminum pans, about 6–10 mg of treated skin samples were deposited. At the same time, an empty pan served as a reference. Skin samples were regularly subjected to heat between 20 and 200 ^{0}C at a rate of 5 degrees per minute. At least three times, each experiment was conducted. The DSC analyzer was calibrated and verified using an indium standard in order to assure data accuracy and reproducibility.[12]

FT-IR experiments

To eliminate evidence of permeation enhancer, the excised rat skin samples were treated for 4 hours with olive oil, corn oil, clove oil, menthol, and eucalyptus oil, then vacuum dried (650 mm

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Hg, 25±1°C) for 30 minutes and kept in desiccators. An FT-IR facility was used to scan the skin samples in the 4000 to 500 cm^{-1} range (Uker, Vertex70, and Germany).[12]

RESULT AND DISCUSSION

Effect of herbal penetration enhancers on mefenamic acid permeability

Table 1, 2 and Figure 1, 2 show the permeability parameters following skin pretreatment with natural enhancers for 2 and 4 hours compared to control, as well as the quantity of mefenamic acid penetrated through the rat abdomen skin from different enhancers. Table 1 demonstrates the impact of natural enhancers' pretreatment for 2 hours on mefenamic acid permeability compared to control as ERflux (drug flux ratio after and before skin pretreatment with enhancer) and ERD (drug flux ratio after and before skin pretreatment with enhancer) (drug diffusion coefficient after and before skin pretreatment with enhancer).[12] According to the findings, eucalyptus oil, olive oil, corn oil, clove oil, and menthol substantially improved mefenamic acid flux and diffusion coefficient, according to the findings. Clove oil increased mefenamic acid flux the most after a 2-hour skin pretreatment, increasing it by up to 7.91-fold compared to control, followed by eucalyptus oil (3.32-fold), olive oil (2.6-fold), corn oil (1.119-fold), and menthol (1.13-fold). Except for corn oil ($p > 0.05$), all of the natural penetration enhancers had a significant influence on the diffusion coefficient ($p < 0.05$), with clove oil having the largest enhancement effect compared to control.

Table 2 illustrates the impact of natural penetration enhancers' pretreatment for 4 hours on mefenamic acid permeability as ERflux and ERD when compared to control. According to the data, eucalyptus oil, olive oil, corn oil, clove oil, and menthol substantially enhance mefenamic acid flux and diffusion coefficient. After a 4-hour skin pretreatment, clove oil increased mefenamic acid flow the most, up to 18.65-fold, compared to control, followed by eucalyptus oil (3.57-fold), olive oil (3.57-flod), corn oil (2.65-flod), and menthol (2.65-flod) (2.17-fold). Except for corn oil ($p > 0.05$), all of the penetration enhancers had a significant impact on the diffusion coefficient, with clove oil having the biggest enhancing effect on the diffusion coefficient compared to control.

1, 8-cineole makes up about 75% of eucalyptus oil. Cineole is a cyclic terpene that makes liquid pools in the SC and changes the stratum corneum's lipid structure. This makes it easier for polar and non-polar medicines to get through the membrane.[20]

Wang and colleagues investigated the impact of corn oil, olive oil, and jojoba oil variations on the increase in skin permeability of aminophylline via the human skin. The data revealed that vegetable oils had a larger role in increasing drug permeability, with jojoba oil having the most significant impact.[21] Salimi and colleagues looked at how different penetration enhancers affected meloxicam's skin permeability. Transcutol oil, eucalyptus oil, and oleic acid were shown to have the greatest impact on skin flux increase.[22] Mohammad Soleymani and colleagues looked at how different penetration enhancers affected adapalene skin permeability. Clove oil and eucalyptus oil were shown to have the greatest impact on skin flux and partition coefficient increase.[23]

Differential Scanning Calorimetry (DSC)

The thermotropic behavior of treated skin was assessed using mean transition temperatures (Tm) and corresponding enthalpies (H). Transition temperatures and enthalpies are shown in Table 3,

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Tm1 and Tm2 from hydrated rat skin were 67.5 °C and 112 °C, which means that the lipids in the skin had melted and the keratin in the skin had been broken down irreversibly. Any decrease in Tm might be the result of lipid breakdown in the bilayer and irreversible protein denaturation in the SC. While lipid fluidization in lipid bilayers and protein–lipid complexes is often linked to a decrease in enthalpy, this is not always the case.[16] In human dermal DSC graphs, Kaushik et al. found three endothermic transition peaks at temperatures of 59-63°C (Tm1), 75-82°C (Tm2), and 99.5–120°C (Tm3).They proposed that Tm1 relates to the change of lipid forms from a lamellar to a disordered state, Tm2 to protein-lipid or the rupture of polar head groups of lipids, and Tm3 to irreversible denaturation of proteins, respectively.[15]

When compared to hydrate rat skin, the thermograms of skin treated with menthol show reduced Tm2 and H1, H2. Tm1 was also eliminated by menthol. In this study, we found that menthol changed the structure of the SC layer in a number of ways, including making lipids more fluid in the intercellular area, breaking down lipids in the bilayer, and permanently breaking down proteins.

The DSC findings from skin pretreatment with eucalyptus oil reveal reduced Tm1 and H1, as well as decreased H2 and Tm2. This means that eucalyptus oil may make the skin more permeable by causing lipids in the bilayer to break down and irreversible protein destabilization in the SC layer.

Tm1 changed to lower melting points and Tm2 rose in skin pretreated with olive oil thermograms, whereas H1 and H2 were reduced compared to controls. This means that olive oil may make the skin more permeable by causing lipids in the bilayer to break down and irreversible protein destabilization in the SC layer.

When skin was treated with corn oil, the melting points of Tm1 and Tm2 changed lower and higher, respectively, compared to the control. In addition, compared to the control, H1 and H2 shifted to lower levels. This suggests that corn oil may increase skin permeability by breaking down lipids in the bilayer and irreversibly destabilizing proteins in the SC layer.

As skin was treated with clove oil, the melting points of Tm1 and Tm2 changed lower and higher, respectively, when compared to the control. In addition, as compared to the control, H1 and H2 shifted to lower levels. A study found that clove oil may make skin more permeable because it breaks down lipids in the bilayer and permanently changes proteins in the SC layer, which makes the skin more permeable.

FT-IR Spectroscopy

Tables 4, 5, and 6 provide spectrum analysis of samples, showing peak position and intensity variations from 4000cm^{-1} to 500cm^{-1}. If the wave number increases (blue shift), it indicates that the SC membrane (lipid bilayer) is becoming more fluid, making it simpler for drugs to enter the body through the SC.[24] On the other hand, lipid groups reorient, causing a change to a lower wave number (e.g., red shift) and strengthening of subcutaneous barrier qualities, resulting in a slowing down of permeant passage through the skin.[11]

The spectra of menthol-treated rat skin show changes in peak height and wave numbers. Red shifts were noticed in skin that had been treated with menthol at wave numbers (2838.63cm^{-1} and

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2747.53cm^{-1}). This shows that lipid groups have been altered, resulting in a stronger SC barrier. There was a relative red shift in 1728. 58 cm^{-1} band was observed in skin pretreated with menthol, indicating the formation of strong hydrogen bonds within the lipid structures. Pretreating skin rats with menthol had a 75.17 percent reduction in the mean peak height of C-N stretching (Amide I) absorbance, showing that it interacts mostly with proteins in the SC layer. Significant reductions in the height of peaks in the 2981.66, 2915.86, 1690.2, and 1642.65 cm^{-1} wave numbers were seen in the FT-IR spectra of skin pretreated with clove oil. According to the findings, clove oil interacts mostly with lipids and proteins in the SC layer. The findings of the permeability parameters after clove oil pretreatment correlate with FT-IR and DSC measurements.

Changes in peak height and wave numbers are seen in the spectra of rat skin prepared with eucalyptus oil. At wave numbers (2848.59 cm-1 and 2741.57 cm^{-1}), red shift was seen in skin prepared with eucalyptus oil, suggesting lipid reorientation that causes SC barrier characteristics to be strengthened. Skin prepared with eucalyptus oil showed a red shift in which the peak number's height (1654.93 cm^{-1}) increased. The wave number (1791.51cm^{-1}) decreases in peak height, causing blue shifts.

Changes in peak height and wave numbers may be seen in the spectra of corn oil-treated rat skin. Skin treated with corn oil showed a blue shift in peak number heights (2981.64, 2919.9, 1733.58, and 1561.21 cm^{-1}), suggesting denaturation of proteins and lipids in the SC layer.

The spectra of rat skin pretreated with olive oil reveal changes in peak height and wave numbers. This implies that when olive oil was used to treat skin, the peak number shifted blue (2981.64, 1733.85 cm^{-1}). This shows that the olive oil broke down proteins and lipids in the SC layer.

CONCLUSION

According to the findings, the permeation enhancers utilized improved drug permeability through excised rat skin. The most plausible mechanisms for greater ERflux, ERD, and ERP ratios were lipid fluidization, disruption of lipid structure, and intracellular keratin irreversible denaturation in the SC by eucalyptus oil, menthol, corn oil, olive oil, and clove oil.

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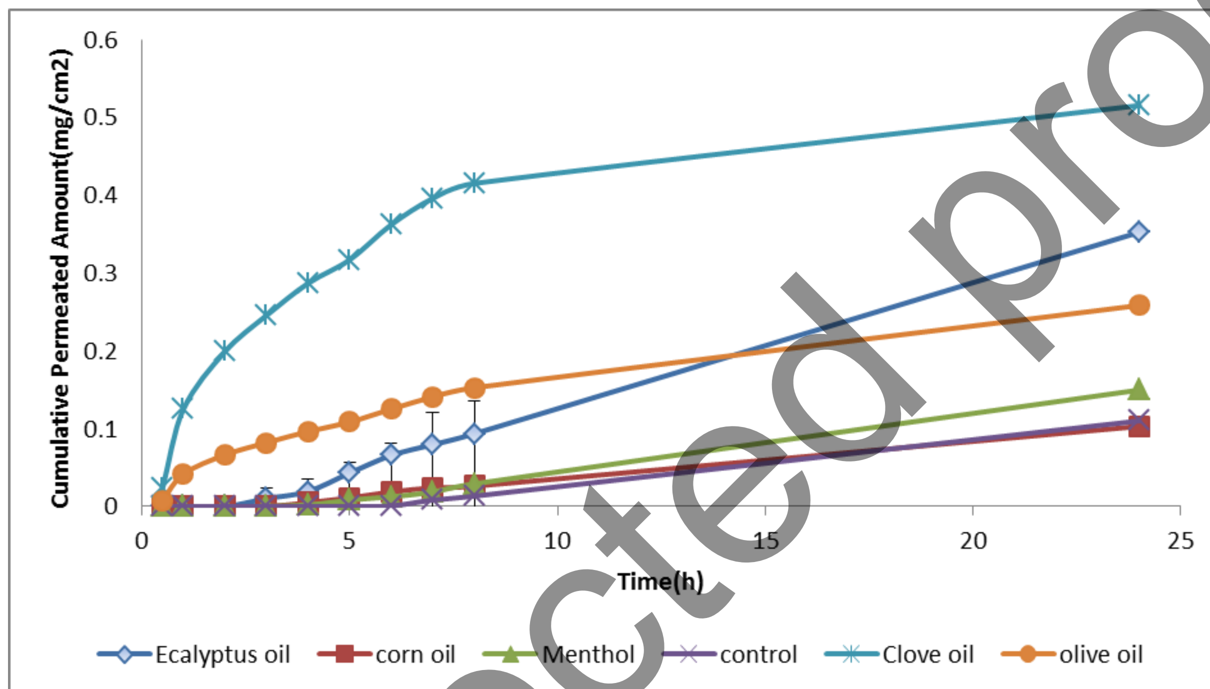

Figure 1. The amount of mefenamic acid permeated after 2 hours pretreatment rat skins with various herbal penetration enhancers

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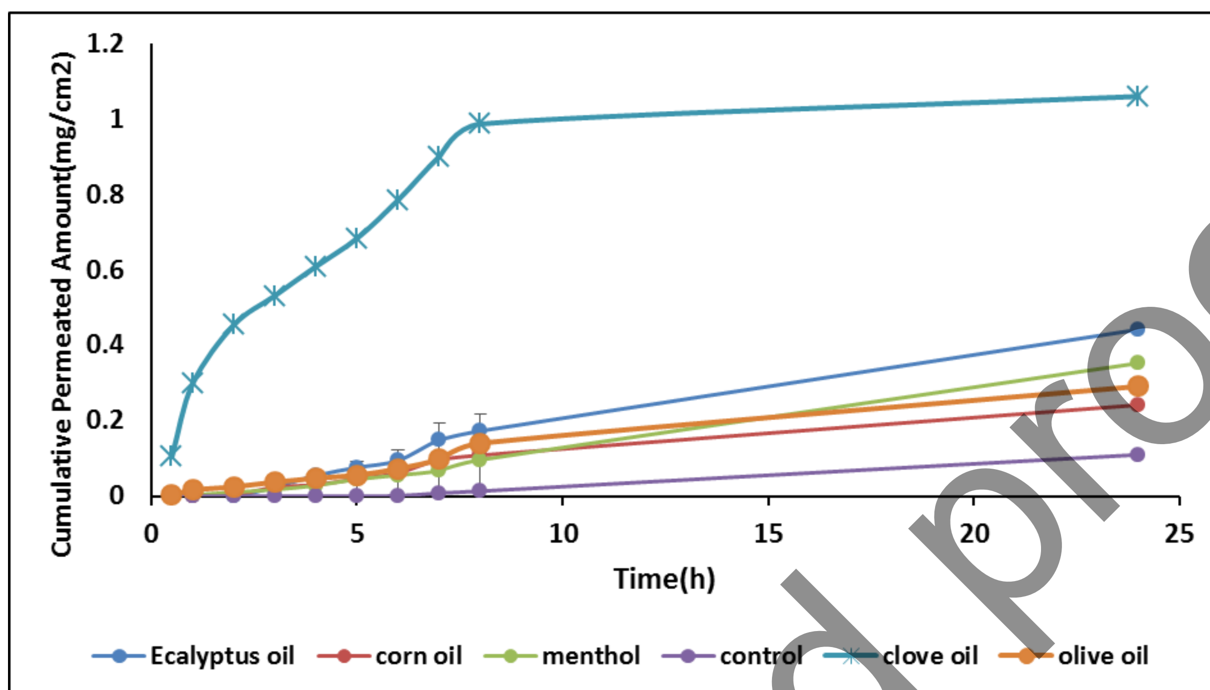

Figure 2. The amount of mefenamic acid permeated after 4 hours pretreatment rat skins with various herbal penetration enhancers

Table 1: Permeability parameters after 2 hours pretreatment with permeation enhancers compared with control (Mean ± SD, n = 3)

Enhancer	**J_{ss}(mg/cm^2.h)**	**D_{app}(cm^2/h)**	**P(cm/h)**	**T_{lag}(h)**	**ERflux**	**ERD**	**ERP**
Control	0.0060±0.00010	0.1090±0.17000	0.0006±0.00001	5.62±0.10	-	-	-
Menthol	0.0067±0.00010	0.0835±0.00200	0.0007±0.00010	4.20±0.22	1.13±0.28	1.12±0.35	1.13±0.28
Eucalyptus oil	0.0190±0.00100	0.0550±0.00600	0.0019±0.00010	2.60±0.62	3.32±1.26	1.51±1.03	3.31±0.26
Olive oil	0.0145±0.00100	0.1405±0.00100	0.0014±0.00020	3.85±0.86	2.60±1.57	3.91±0.95	2.60±0.57
Corn oil	0.0069±0.00100	0.0826±0.01100	0.0007±0.00020	3.30±0.60	1.199±0.35	1.46±0.70	1.20±0.35
Clove oil	0.0460±0.0003	0.1103±0.00800	0.0046±0.00030	1.93±0.01	7.91±0.80	6.24±0.59	7.90±0.06

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Table 2: Permeability parameters after 4 hours pretreatment with permeation enhancers compared with control (Mean ± SD, n = 3)

Enhancer	**J_{ss}(mg/cm^2.h)**	**D_{app}(cm^2/h)**	**P(cm/h)**	**T_{lag}(h)**	**ERflux**	**ERD**	**ERP**
Control	0.0060±0.0001	0.1093±0.017	0.0006±0.00001	5.61±0.10	-	-	-
Menthol	0.0126±0.0001	0.4095±0.002	0.0012±0.00010	1.42±0.50	2.17±0.68	36.11±0.68	2.17±0.68
Eucalyptus oil	0.0208±0.0010	0.1808±0.002	0.0020±0.00100	2.85±0.70	3.57±0.64	5.78±0.06	3.57±0.64
Olive oil	0.0206±0.0020	0.4976±0.030	0.0020±0.000100	0.30±0.03	3.63±0.56	49.83±0.64	3.63±0.56
Corn oil	0.0158±0.0010	0.0627±0.004	0.0015±0.00100	1.74±0.60	2.65±0.20	2.82±0.66	2.65±0.20
Clove oil	0.1098±0.0500	0.0337±0.003	0.0110±0.00200	4.1±0.90	18.35±0.92	1.55±0.05	18.35±0.40

Table 3: Effect of permeation enhancer on the thermal properties of Excised rat skin (mean ± SD, n = 3).

Enhancer	Penetration Enhancer	Transition temperature (^{0}C)	Transition enthalpy (mj/mg)	
	Tm_1	Tm_2	H1	H2
Water(control)	67.5±2.1	112.0±6.6	7.010±0.4	552.4±9.0
Menthol	0	124.0±0.1	0	2.7±0.3
Eucalyptus oil	31.0±0.9	127.5±1.1	2.672±0.1	8.9±0.8
Olive oil	37.7±0.9	115.0±0.9	5.844±0.6	6.2±0.2
Corn oil	0	118.1±1.1	0	99.9±2.1
Clove oil	36.0±0.2	116.0±0.5	0.900±0.2	2.2±0.1

Tm_1= mean transition temperature of lipids; SC **Tm_2**= mean transition temperature of irreversible denaturation of intracellular SC keratin; **H_1**= transition enthalpy of lipid phase SC **H_2**= transition enthalpy of keratin phase SC

Table 4. FT-IR Peak wave numbers (cm^{-1}) changes compared to control (untreated skin) and abdominal hydrated whole skin rat following treatment with different enhancers. (mean±SD, n=3)

Enhancer	C-H stretching Asy	C-H stretching Sym	C=O stretching of lipid ester	AmideI	AmideII
water	2981.77±0.16	2856.34±0.16	1731.68±0.14	1667.04±0.12	1547.67±0.11

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menthol	2838.63±0.15	2747.53±0.13	1728.58±0.11	1603.87±0.16	1538.91±0.20
Eucalyptus oil	2848.59±0.12	2741.57±0.15	1791.51±0.13	1654.93±0.18	1565.24±0.12
Olive oil	2990.78±0.12	2887.85±0.14	1743.31±0.18	1626.18±0.18	1549.92±0.15
Corn oil	2981.64±0.21	2919.9±0.14	1733.85±0.19	1647.25±0.13	1561.27±0.14
Clove oil	2981.66±0.15	2915.86±0.11	-------------	1690.2±0.21	1642.65±0.17

Table 5. Decrease in mean peak height (± SD), compared with control (hydrated skin) of C=O stretching (Amide I) and C-N stretching of keratin (Amide II) absorbance of abdominal hydrated whole skin rat following treatment with different enhancers (Mean ± SD, n=3)

Enhancer	Asymmetric C-H stretching		Symmetric C-H stretching		C=O stretching of lipid ester	
	Wave number	%D	Wave number	%D	Wave number	%D
water	1.8355±0.008	-----	1.95±0.005	-	2.061±0.001	-
menthol	0.527±0.050	71.29	0.512±0.003	73.74	2±0.01	2.96
Eucalyptus oil	0.517±0.010	71.83	0.436±0.008	77.64	0.775±0.006	62.40
Olive oil	0.381±0.001	79.24	0.207±0.010	89.38	0.425±0.002	79.38
Corn oil	2.272±0.005	N.D	2.323±0.007	N.D	2.079±0.005	N.D
Clove oil	1.227±0.004	33.15	0.464±0.010	76.21	0	100.00

%Decrease in peak height (%D) = (peak height from untreated whole skin-peak height from solvent treated whole skin)/ peak height from untreated whole skin x 100
*N.D = No Decrease in peak height

Table 6. Decrease in mean peak height (± SD), compared with control (hydrated skin) of C=O stretching (Amide I) and C-N stretching of keratin (Amide II) absorbance of abdominal hydrated whole skin rat following treatment with different enhancers (Mean ± SD, n = 3)

Enhancer	Amide1 stretching of keratin		Amide2 stretching of keratin	
	Wave number	%D	Wave number	%D
water	2.111±0.006	-	2.151±0.005	-
menthol	1.2±0.009	43.15	0.534±0.009	75.17
Eucalyptus oil	0.727±0.005	65.56	0.727±0.007	66.20
Olive oil	0.479±0.010	77.31	0.611±0.010	71.59
Corn oil	2.1±0.006	0.52	2.04±0.011	5.16
Clove oil	1.889±0.020	10.52	1.94±0.006	9.81

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