ORIGINAL ARTICLE

Terpenes: Effect of lipophilicity in enhancing transdermal delivery of alfuzosin hydrochloride

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J. Adv. Pharm. Tech. Res.

ABSTRACT

Transdermal drug delivery has attracted much attention as an alternative to intravenous and oral methods of delivery. But the main barrier is stratum corneum. Terpenes classes of chemical enhancers are used in transdermal formulations for facilitating penetration of drugs. The aim of the study is to evaluate terpenes as skin penetration enhancers and correlate its relationship with permeation and lipophilicity. In this study, alfuzosin hydrochloride (AH) hydrogels were prepared with terpenes using Taguchi orthogonal array experimental design. The formulations contained one of eight terpenes, based on their lipophilicity (log P 2.13-5.36). The percutaneous permeation was studied in rat skin using diffusion cell technique. Flux, cumulative amount, lag time and skin content of AH were measured over 24 hours and compared with control gels. Nerolidol with highest lipophilicity (log P 5.36 ± 0.38) showed highest cumulative amount (Q_{24}) of 647.29 ± 18.76 μg/cm^2 and fluxrateof 28.16 ± 0.64 μg/cm^2/hour. It showed decreased lag time of 0.76 ± 0.15 hours. Fenchone (2.5%) (log P 2.13 ± 0.30) produced the longest lag time 4.8 ± 0.20 hours. The rank order of enhancement effect was shown as nerolidol > farnesol > limonene > linalool > geraniol > carvone > fenchone > menthol. Lowest skin content was seen with carvone. Increase in lipophilicity of terpenes showed increase in flux, cumulative amount (Q_{24}), and enhancement ratio which was significant with $P < 0.000$. But lag time was decreased and no correlation was found between lipophilicity and skin content. Histological studies showed changes in dermis which can be attributed to disruption of lipid packing of stratum corneum due to effect of nerolidol within lipid lamellae. It was found that small alcoholic terpenes with high degree of unsaturation enhance permeation of hydrophilic drugs, liquid terpenes enhance better than solid terpenes and terpenes with high lipophilicity are good penetration enhancers.

Key Words: Alfuzosin hydrochloride, lipophilicity, taguchi robust design method, terpenes, transdermal permeation

INTRODUCTION

Transdermal delivery is more advantageous over conventional modes of drug administration. It is

- convenient,
- bypasses first-pass metabolism,
- provides a steady-state plasma concentration of the drug,
- provides long term therapy in a single dose
- and improves patient compliance.

However, the skin permeation of clinically useful drugs is generally poor with some exception (it has small molecular weight (< 300 Da) and lipophilic nature) because the stratum corneum functions as a barrier against foreign substances.[1] The most widely implemented approach to overcome this skin barrier has been the use of chemical penetration enhancers, which ideally alter the physiochemical nature of the stratum corneum safely and reversibly to facilitate the drug's delivery through the skin.[2] According to the lipid-protein-partitioning theory, penetration enhancers

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Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/2231-4040.104712

may increase the permeability of a drug by affecting the intercellular lipids of the stratum corneum via extraction or fluidization and/or by increasing the partitioning of the drug in the stratum corneum membranes, and/or by changing conformations within the keratinized protein component.[3]

Terpenes classes of chemical enhancers are used in transdermal formulations for facilitating penetration of drugs.[4] Terpenes which are derived from plant essential oils are naturally occurring hydrocarbons based on combinations of the isoprene units.[5] They were reported to have less toxicity, high percutaneous enhancement abilities and low cutaneous irritancy at low concentrations (1-5%).[6] The effect of a specific terpene on skin depends upon its chemical structure and physicochemical properties, such as its lipophilicity, size and chiralty, boiling point and energy of vaporization and degree of unsaturation.[7] Terpenes can increase skin permeation by one or more of the mechanisms: interacting with stratum corneum lipids and/or keratin, and increasing the solubility of drug into stratum corneum lipids.[8]

Alfuzosin hydrochloride (AH) an alpha adrenoreceptor antagonist used for symptomatic relief of benign prostatic hyperplasia requires long term therapy in place of surgery. AH has molecular weight (425.9 daltons), log P (1.604), melting point (225-235°C), dose (2.5 to 10mg per day), half-life (3-5 hours) and undergoes hepatic metabolism to inactive metabolites. As AH possesses properties required for transdermal delivery and as it requires long term therapy, so it was aimed for transdermal delivery.[9]

Taguchi robust design is a statistical technique which studies all levels of input parameters with fewer experiments and optimizes the experiment having least variability. The variability of a property is expressed by signal to noise ratio (S/N ratio) and the experiment having maximum S/N ratio is considered as optimum condition.[10]

In the present study the influence of lipophilicity on permeation was studied by selecting eight terpenes of different lipophilicities. These terpenes were incorporated in hydrogels and their efficiency of permeating AH was optimized using Taguchi robust design method.

MATERIALS AND METHODS

Materials

Alfuzosin hydrochloride (AH) was obtained as a gift sample from Dr. Reddy's Laboratories Ltd (Hyderabad, India). Acrypol-980 was purchased from Corel Pharma Ltd. (Ahmedabad, India). Nerolidol, farnesol, limonene, linalool, geraniol, carvone, fenchone and menthol were purchased from Alfa Aesar Ltd (USA). Propanol, glycerin and triethanolamine were purchased from S. D. Fine-Chem. Ltd. (India).

Preparation of Gels

AH gels were prepared using acrypol 980 (2%), propanol (5%), glycerin (5%), terpene (2.5, 5%), triethanolamine and distilled water (up to 100ml). Propanol, glycerin and water were mixed together and the mixture was divided into two equal parts. Acrypol 980 was added to one part and soaked for 1 hour. Drug AH (1%) and terpene was added to the other part and this solution was added to acrypol solution. Appropriate amounts of triethanolamine was added to the solution and mixed until the gel was formed. Terpenes were selected based on their lipophilicity given in Table 1. Terpene was added according to Taguchi L16 orthogonal array experimental design given in Table 2.

Experimental Design

Taguchi L16 orthogonal array experimental design was constructed with type of terpene and concentration of terpene as independent variables. Cumulative amount permeated in 24 hours (Q_{24}) and flux was selected as dependent variables. The effect of independent variables on dependent variables was studied using SN ratio plots. MINITAB 16 software (Minitab Inc., PA, USA) was used for the generation and evaluation of the statistical experimental design.[11]

Solubility Studies

Saturated solubility of AH was evaluated by adding excess of drug to 10ml of propanol, glycerin and water (5:5:90) including appropriate quantity of terpene enhancer. The suspension was shaken using rotary shaker for 24 hours at room temperature, later it was centrifuged for 15 minutes at 3000 rpm, filtered and diluted with the vehicle and AH concentration was analyzed by UV-VIS double beam spectrophotometer (Chemito Spectrascan UV2600, India) at 245nm. The effect of terpene was determined by enhancement ratio which was calculated by dividing the solubility of AH in terpene to the solubility in control (no terpene).

Ex Vivo Permeation Studies

The experimental protocol was approved by the institutional animal ethical committee (IAEC) (Reference number: 320/CPCSEA).

Male Wistar rats (150-180 g) were used for permeation

Table 1: Log P of terpenes used as enhancers

Terpene	Log P
Nerolidol	5.36±0.38
Farnesol	5.31±0.34
Limonene	4.58±0.23
Linalool	3.28±0.27
Geraniol	3.18±0.30
Carvone	2.23±0.25
Fenchone	2.13±0.30
Menthol	3.20±0.19

Table 2: Taguchi L16 orthogonal array experimental design with independent variables and observed responses

Formulation code	Independent variables		Dependent variables	
	A	B (%)	Y_1(μg/cm²)	Y_2(μg/cm²/hr)
TA1	1 (Nerolidol)	1 (2.5)	561.02±7.81	25.14±0.18
TA2	1 (Nerolidol)	2 (5)	647.29±18.76	28.16±0.64
TA3	2 (Farnesol)	1 (2.5)	513.70±9.65	22.48±0.28
TA4	2 (Farnesol)	2 (5)	566.55±7.49	27.58±0.32
TA5	3 (Limonene)	1 (2.5)	473.80±8.99	21.13±0.70
TA6	3 (Limonene)	2 (5)	517.11±10.08	22.25±0.19
TA7	4 (Linalool)	1 (2.5)	442.68±6.79	18.96±0.11
TA8	4 (Linalool)	2 (5)	474.73±6.37	20.96±0.09
TA9	5 (Geraniol)	1 (2.5)	398.52±9.55	18.49±0.30
TA10	5 (Geraniol)	2 (5)	438.20±8.22	19.08±0.16
TA11	6 (Carvone)	1 (2.5)	386.49±6.60	17.51±0.23
TA12	6 (Carvone)	2 (5)	401.77±10.97	17.25±0.27
TA13	7 (Fenchone)	1 (2.5)	335.32±6.50	15.31±0.13
TA14	7 (Fenchone)	2 (5)	387.28±7.50	17.17±0.27
TA15	8 (Menthol)	1 (2.5)	316.81±5.04	14.31±0.14
TA16	8 (Menthol)	2 (5)	365.65±6.63	16.24±0.21

A, type of terpene, B, concentration of terpene (%), Y_1, cumulative amount permeated in 24 hours, Q_{24} (μg/cm²), Y_2, flux, J_{ss}, (μg/cm²/hr), Values represent mean±SD (n=3)

study. The animal was sacrificed by excessive ether anesthesia and hair was removed on abdomen using an animal hair clipper (Aesculap, Germany). Abdominal skin section was excised and observed for existence of cuts and wounds. The fat adhering on dermis was removed using scalpel and finally it was washed under tap water. The skin was stored at −20°C and used within a week.

For the permeation studies locally fabricated Keshary-Chein diffusion cells with an area of 4.9 cm² and 20ml receptor volume were used. The thawed rat skin was mounted onto diffusion cell such that the dermis side was in constant contact with receptor solution. 500mg of gel was applied to the stratum corneum facing the donor compartment and the hydrodynamics in the receptor compartment were maintained by stirring on magnetic stirrer at 600 rpm (Remi Equipments Ltd.). 1ml sample was withdrawn at predetermined time intervals for 24 hours and drug content was analyzed by UV-VIS double beam spectrophotometer (ChemitoSpectrascan UV2600, India) at 245nm.[12]

After 24 hours study drug retained in the skin was determined. For skin content studies, after study the skin was removed, washed with methanol and homogenized. The mixture was centrifuged at 7000 rpm for 30 minutes, filtered and analyzed for drug content spectrophotometrically at 245 nm.

Histopathological Studies

To determine the effect of terpene on skin, histopathological studies were conducted according to the protocol approved by institutional animal ethical committee (IAEC) (Reference number: 320/CPCSEA). The formulations placebo gel (without terpene) and optimized gel formulation (with terpene) were applied to wistar rats (hair was removed at application sites) for 6 hours. Then the animal was sacrificed by excessive ether anesthesia and application site was excised, stored in neutral formalin solution (50%). It was further subjected to histological processing such as dehydration and rehydration with alcohols, staining with Hematoxylin-eosin dye, paraffin blocks and slide preparation. H and E slides were evaluated using dark-light microscope by a blinded assessor.

Data Analysis

The cumulative amount permeated in 24 hours (Q_{24}) was calculated from permeation studies. Flux (J_{ss}) was calculated from slope of curve on plotting Q_{24} Vs time and X-intercept of straight –line portion of the curve is Lag time. Flux divided by donor concentration resulted in apparent permeability coefficient (K_p).[13] Mean and standard deviation were calculated using Microsoft Excel 2003. The experiments were performed in triplicate ($n = 3$) and data was subjected to one-way ANOVA at a significance level of $P \leq 0.05$ using MINITAB 16 software (Minitab Inc., PA, USA).

RESULTS AND DISCUSSION

Hydrogels of AH with terpenes as enhancers were prepared according to the formulations given by Taguchi L16 orthogonal array experimental design. Two factors mixed level with eight levels for factor one and two levels for factor two were selected as independent variables and responses, cumulative amount permeated in 24 hours and flux were

selected as dependent variables for construction of Taguchi L16 orthogonal array experimental design. The formulations were optimized using SN ratio plots by Taguchi robust design method.

Effect of Terpene Type

Eight terpenes of different lipophilicities were selected as eight levels of factor, terpene type. Lipophilicities of decreasing order were selected for eight levels so nerolidol with log P 5.36 ± 0.38 was considered as first level and fenchone with log P 2.13 ± 0.30 as seventh level. Menthol with log P 3.20 ± 0.19 was considered as eight level even though its log P is greater than geraniol (log P 3.18 ± 0.30) as it is a solid terpene and researchers have reported liquid terpenes permeate better than solid terpenes.[14] To study the effect of physico-chemical properties of terpenes on permeation, such as lipophilicity, physical nature etc., different terpenes were selected. From the main effects SN Ratio plots shown in Figures 1a and 2a it can be observed that type of terpene had a significant effect on cumulative amount permeated in 24 hours and flux ($P < 0.005$). As terpenes was selected based on decreasing order of lipophilicity, the permeated amount and flux also decreased showing a linear relationship between permeation and lipophilicity proving the influence of lipophilicity on permeation. Menthol a solid terpene of higher lipophilicity than carvone and fenchone enhanced permeation lesser than carvone and fenchone which shows liquid terpenes permeate better than solid terpenes. Of the eight terpenes Nerolidol with highest lipophilicity showed maximum permeation.

Effect of Concentration

Two concentrations 2.5% and 5% were selected as two levels. Each terpene was evaluated at these two concentrations. SN Ratio plots shown in Figures 1b and 2b indicate the effect of concentration on permeation was not significant ($P > 0.05$). But it was observed with each terpene as concentration increased the permeated amount also increased.

Solubility of AH in solvent mixture (propanol: glycerine: water (5:5:90)) of control gel was represented as unity [Table 3]. Major enhancement of solubility was not observed with terpenes. Nerolidol (5%) showed maximum enhancement of solubility by 1.23 fold followed by geraniol (2.5%) by 1.21 fold, fenchone (5%) and limonene (5%) by 1.18 fold, nerolidol (2.5%) by 1.17 fold, geraniol (5%) and carvone (5%) by 1.13 fold, limonene (2.5%) by 1.10 fold. Fenchone (2.5%) and farnesol (5%) showed similar solubility as control gel solvent mixture. But solubility was decreased by menthol (2.5 and 5%), carvone (2.5%), linalool (2.5 and 5%) and farnesol (2.5%). No significance was observed in the enhancement of solubility with lipophilicity of terpenes ($P > 0.05$).

The permeability coefficient (K_p), lag time, skin content and enhancement ratio of formulations with different terpenes as enhancers are listed in Table 3.

Maximum permeability coefficient (K_p) of 5.63 ± 0.13 cm/hour was obtained with Nerolidol 5% followed by farnesol 5% (5.51 ± 0.67 cm/hour), nerolidol 2.5% (5.03±0.04 cm/hour), farnesol 2.5% (4.50 ± 0.06 cm/hour), limonene 5% (4.44 ± 0.03 cm/hour) etc. The lowest K_p 2.86 ± 0.03 cm/hour was obtained with menthol 2.5%.

Nerolidol 5% showed lowest lag time of 0.76 ± 0.15 hours when compared with control (2.96 ± 0.35 hour). The highest lag time was observed with fenchone 2.5% (4.8 ± 0.20 hour) followed by fenchone 5% (4.6 ± 0.10 hour).

The amount of drug retained in skin after 24 hours study was calculated as skin content. Skin content of 1256.58 ± 64.39µg/g was obtained with control [Table 3]. When compared with control less amount of drug was retained in skin with terpenes. The lowest skin content was obtained with carvone 2.5% (80.85 ± 9.46 µg/g) next by carvone 5% (92.09 ± 17.52 µg/g). The highest skin content among terpenes was obtained with linalool 2.5% (408.67 ± 13.01 µg/g) and linalool 5% (387.25 ± 14.66 µg/g). With Nerolidol 2.5% and 5% (240.96 ± 9.35µg/g, 298.89 ± 14.00 µg/g) were obtained respectively. The effect of terpene enhancers on skin content was previously studied by Williams and Barry using differential scanning

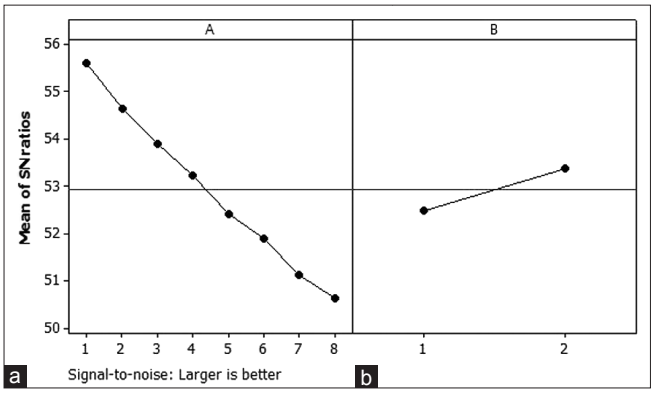

Figure 1: Mean effects plot for SN Ratios of (a) type of terpene and (b) terpene concentration for cumulative amount permeated in 24 hours

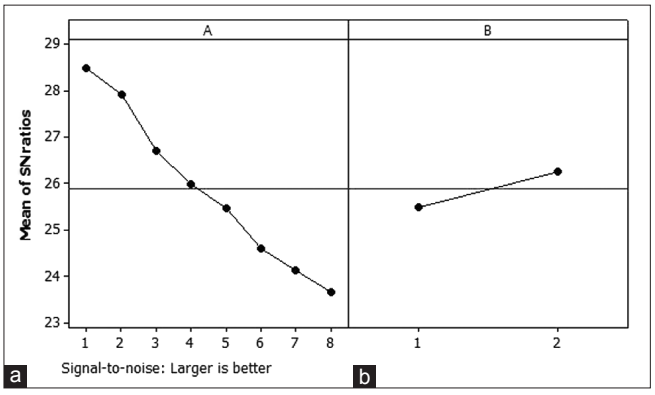

Figure 2: Mean effects plot for SN Ratios of (a) type of terpene and (b) terpene concentration for flux

calorimetry (DSC). They have observed that no correlation exists between DSC results and enhancing abilities of the terpenes.[15]

The highest cumulative amount permeated in 24 hours (Q_{24}) was obtained with nerolidol 5% (647.29 ± 18.76 µg/cm^2) followed by farnesol 5% (566.55 ± 7.49 µg/cm^2), and the lowest Q_{24} among 5% concentration of terpenes was obtained with menthol 5% (365.65 ± 6.63 µg/cm^2) [Table 2]. The permeation profile with 2.5% and 5% terpenes is shown in Figures 3 and Figure 4 respectively.

With 2.5% terpenes concentration maximum permeation was obtained with Nerolidol followed by farnesol. Highest Q_{24} and flux was obtained again with Nerolidol (561.02 ± 7.81 µg/cm^2, 25.14 ± 0.18µg/cm^2/hour) followed by farnesol (513.70 ± 9.65 µg/cm^2, 22.48 ± 0.28µg/cm^2/hour).With 5% concentration also highest flux was obtained with Nerolidol (28.16 ± 0.64µg/cm^2/hour) and farnesol (27.58 ± 0.32µg/cm^2/hour). Nerolidol and farnesol possessed considerably higher log *P* values than the others and hence, enhanced permeation maximum when compared with others.

Comparing with control gel, permeation was enhanced by 3.57 fold by Nerolidol 5% followed by 3.49 fold by farnesol 5%, 3.19 fold by Nerolidol 2.5%, 2.85 fold by farnesol 2.5%. The lowest enhancement was seen with menthol 2.5% by 1.81 fold. The order of enhancement was nerolidol> farnesol>limonene >linalool >geraniol>carvone>fenchone> menthol. The activity of terpenes is related to their chemical structure and other factors such as lipophilicity, size and chiralty, boiling point and energy of vaporization, and degree of unsaturation. Terpenes with high lipophilicity, small size, low boiling point and low energy of vaporization are good sorption enhancers. Small alcoholic terpenes with high degree of unsaturation enhance permeation of hydrophilic drugs.[7] It has been reported from previous studies that hydrophilic terpenes enhance permeation of hydrophilic drugs more effectively than hydrophobic terpenes.[16-19] Nerolidol has been reported as an effective enhancer for nicardipine hydrochloride, carbamazepine,[5] tamoxifen,[20] hydrocortisone[21] and diclofenac sodium.[19] Nerolidol also enhanced the permeation of 5-fluorouracil and its enhancement was reported by Cornwell and Barry[22] that its amphiphilic structure suitable for aligning within lipid lamellae attributed to disruption of highly organized lipid packing of stratum corneum.

Research studies have suggested properties for an ideal terpene enhancer such as they should be hydrophobic and liquid nature, should not be triterpene or tetraterpene and posses ester or aldehyde group.[14] Mono- and sesquiterpenes are better enhancers than di-, tri-, and tetra terpenes as their enhancement is attributed to increasing drug diffusivity within stratum corneum.[23,24] In the present study terpenes studied were mono-and sesquiterpenes; hence, permeation was enhanced when compared with control. Terpenes selected are liquid in nature except menthol which is solid in nature. In the present study least enhancement was observed with menthol even though it is more lipophilic than carvone and fenchone, and in possessing an alcohol group which favors enhancement of hydrophilic drugs than ketone groups. This can be due to solid nature of menthol.

To study the effect of lipophilicity, log P of terpene was

Table 3: Solubility data and permeation parameters of AH formulations

Formulation code	Solubility[a] (mg/ml)	ER_{sol}[b]	Permeability coefficient ($\times 10^{-03}$) (cm/hr)	Lag time (hr)	Skin content (µg/g)	ER[c]
Control	23.34±1.07	1	1.51±0.05	2.96±0.35	1256.58±64.39	1
TA1	27.41±1.34	1.17	5.03±0.04	1.3±0.30	240.96±9.35	3.19
TA2	28.72±1.84	1.23	5.63±0.13	0.76±0.15	298.89±14.00	3.57
TA3	20.75±1.50	0.88	4.50±0.06	1.5±0.20	191.33±16.86	2.85
TA4	24.47±1.67	1.04	5.51±0.67	1.0±0.20	343.74±13.81	3.49
TA5	25.75±1.64	1.10	4.23±0.14	2.03±0.25	318.03±6.84	2.68
TA6	27.58±1.82	1.18	4.44±0.03	1.76±0.15	258.49±12.98	2.82
TA7	13.56±1.00	0.58	3.79±0.02	2.5±0.20	408.67±13.01	2.40
TA8	18.59±1.16	0.79	4.18±0.02	2.4±0.20	387.25±14.66	2.65
TA9	28.27±1.07	1.21	3.70±0.06	2.96±0.15	271.05±12.87	2.34
TA10	26.60±1.91	1.13	3.82±0.03	2.66±0.15	302.23±17.5	2.42
TA11	22.30±1.91	0.95	3.50±0.05	3.16±0.25	80.85±9.46	2.22
TA12	26.4±2.15	1.13	3.45±0.06	3.0±0.20	92.09±17.52	2.18
TA13	23.38±1.12	1.00	3.03±0.03	4.8±0.20	310.13±11.60	1.92
TA14	27.54±2.11	1.18	3.43±0.06	4.6±0.10	329.79±12.33	2.17
TA15	9.95±1.31	0.42	2.86±0.03	4.53±0.15	372.57±11.07	1.81
TA16	12.50±1.07	0.53	3.24±0.04	3.80±0.17	350.57±13.54	2.06

[a]Solubility is solubility of AH in the hydrogel solvent mixture (control) at 25°C, [b]ER_{sol} is enhancement ratio of AH solubility over control solubility, [c]ER is the enhancement ratio of flux of terpenes over control, Values represent mean±S.D (n=3)

correlated with percutaneous parameters of AH. Log P of terpenes greatly influenced Q_{24} and flux. The correlation coefficient for Q_{24} with terpenes 2.5% and 5% is r^2=0.772, P<0.0001 and r^2=0.824, P<0.0001 [Figure 5] respectively and for flux with terpenes 2.5% is r^2=0.764, P<0.0001, 5% is r^2=0.871, P<0.0001 [Figure 6] indicating linear relationship with log P of terpenes at both 2.5% and 5% concentration.

With lag time correlation coefficient for 2.5% is r^2= −0.7195, P<0.0001 and 5% is r^2= −0.7921, P < 0.0001 [Figure 7] respectively indicating a significant correlation between log P of terpenes and lag time.

With skin content coefficient values r^2=0.077 for 5% and r^2=0.000 for 2.5% indicated an insignificant correlation [Figure 8].

In the present study lipophilicity of enhancer played a major

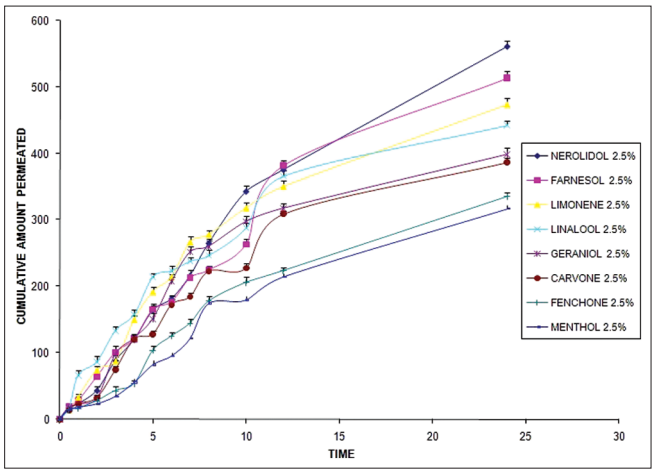

Figure 3: Permeation profile of 2.5% concentration of different terpenes in gel formulations

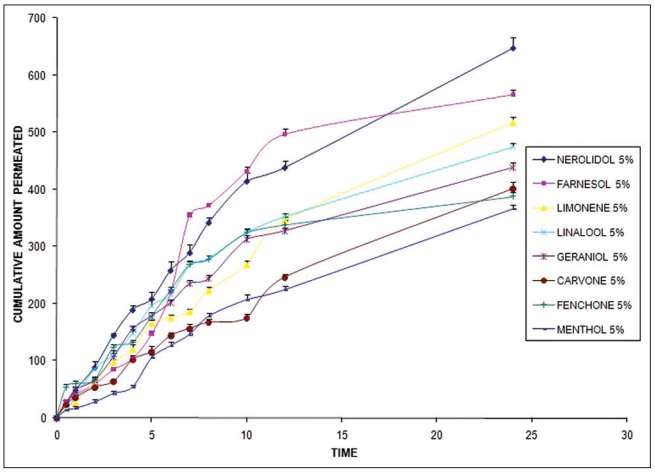

Figure 4: Permeation profile of 5% concentration of different terpenes in gel formulations

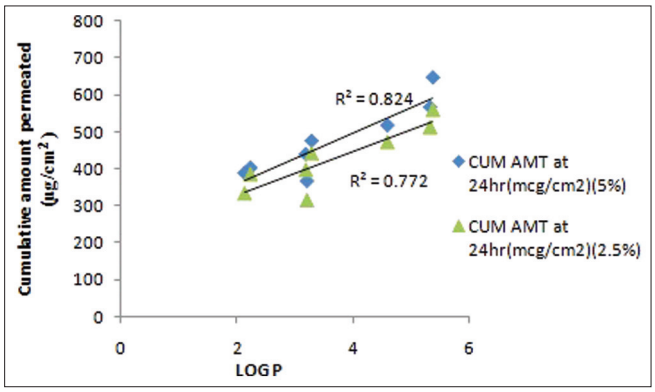

Figure 5: Relationship between Log P of terpenes and cumulative amount permeated in 24 hours

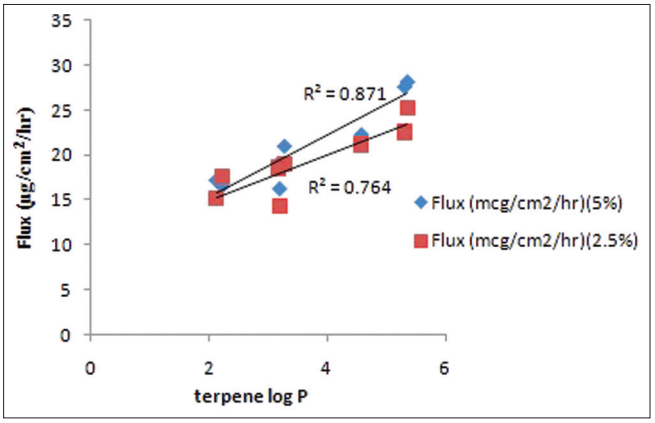

Figure 6: Relationship between Log P of terpenes and flux

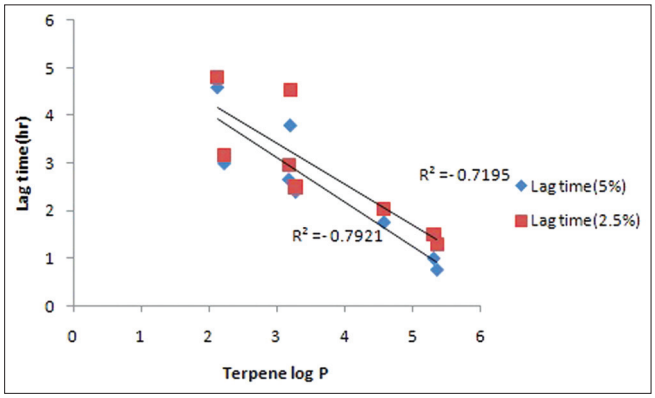

Figure 7: Relationship between Log P of terpenes and lag time

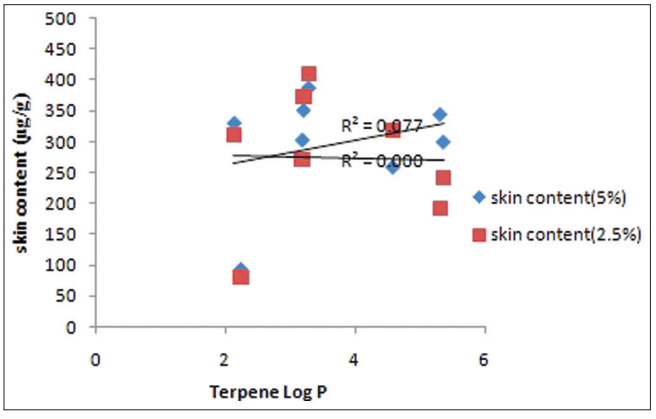

Figure 8: Relationship between Log P of terpenes and skin content

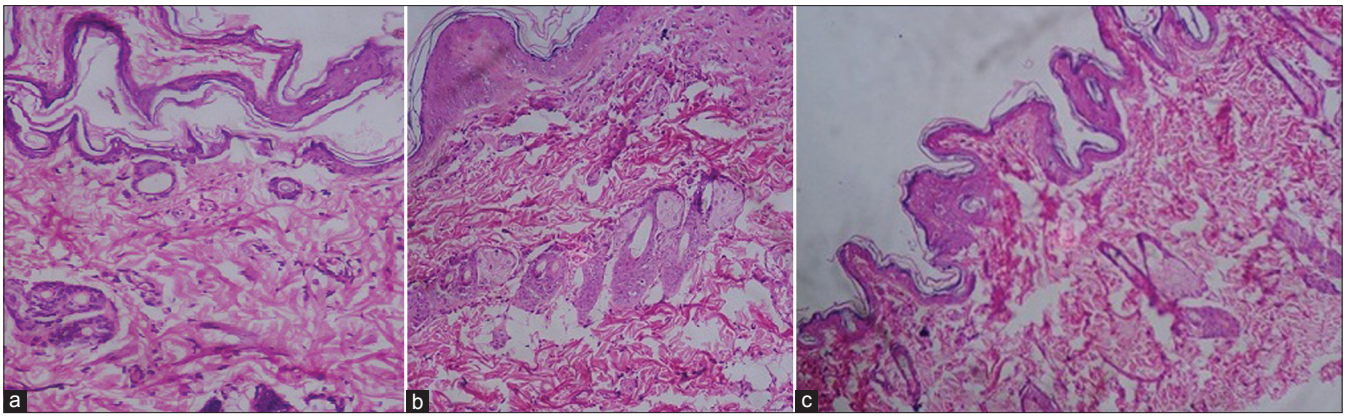

Figure 9: Histological section of skin a) control b) treated with placebo gel (without terpene) and c) treated with TA2 formulation

role and a linear relationship was observed which was also reported by Williams and Barry with 5-fluorouracil,[25] Kang *et al*.,[14] El-Kattan *et al*.[21] and Rio-Sancho *et al*.[26]

H and E sections of skin when observed under 200× magnification in dark field light microscope showed hemorrhage and degeneration in dermis of skin treated with placebo gel (control formulation). Skin treated with optimized formulation TA2 showed presence of mononuclear cell infiltration, mild to moderate congestion, degeneration and fatty change in dermis. Degeneration and fatty change in dermis can be attributed to disruption of highly organized lipid packing of stratum corneum due to alignment of amphiphilic structure of nerolidol within lipid lamellae of stratum corneum. The changes observed in the skin samples showed the action of terpene on lipids and no local toxicity was seen. Photomicrographs of skin sections are shown in Figure 9.

CONCLUSION

A linear relationship was established between log P of terpenes and Q_{24} and flux of AH. As the concentration of each terpene increased the Q_{24} and flux of AH also increased. From Taguchi Robust Design method TA2 formulation containing Nerolidol 5% was optimized which enhanced permeation by 3.57 fold. The effect of terpenes on solubility enhancement ratio was not significant attributing enhancement to diffusion and partitioning of drug into skin and disrupting lipid bilayer of stratum corneum which was supported with histological studies.

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How to cite this article: Prasanthi D, Lakshmi PK. Terpenes: Effect of lipophilicity in enhancing transdermal delivery of alfuzosin hydrochloride. J Adv Pharm Tech Res 2012;3:216-23.

Source of Support: Nil, **Conflict of Interest:** Nil.