TO STUDY THE IN VITRO EFFECT OF SOME ALCOHOL CONTAINING VOLATILE OILS AS THE SKIN PENETRATION ENHANCERS OF CURCUMIN

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Abstract: The effect of geraniol, linalool and nerol on the skin permeation of curcumin was investigated. The mechanism of skin permeation enhancement of curcumin by essential oils treatment was evaluated by Activation energy measurement. The effectiveness of the oils as the penetration enhancers was found to be in the following descending order: geraniol>linalool>nerol. The results of thermodynamic studies and of geraniol incorporated group suggested additional mechanisms for geraniol facilitated permeation. To conclude geraniol has been diagnosed to be the best penetration enhancer of all others to allow curcumin pass through the skin epidermis.

Keywords: Geraniol, linalool, nerol, TDDS

1. Introduction
Curcumin, an extract from the Indian spice turmeric, has been investigated in a variety of human cancers including pancreatic, prostate, breast, head and neck cancer. The first published report demonstrating the topical use of curcumin in cancer reported a sustainable reduction in lesion size and pain.[1] Curcumin has antioxidant, anti-inflammatory, antiangiogenic and anticarcinogenic activity, although its clinical use is limited by low bioavailability.[2] More recently, several studies have examined curcumin’s effect in inhibiting skin carcinogenesis. Additionally, numerous reports have identified signalling pathways related to epidermal growth factor receptors (EGFR) that are essential to formation and progression of cutaneous malignancy. The MTOR and MEK/ERK signalling cascades are two of the most well-studied pathways.[3] In a prior study by our group we subcutaneously injected immunodeficient mice with SRB12-p9 skin SCC and demonstrated that curcumin administered orally significantly inhibited tumor growth and down-regulated pS6, a well-established downstream biomarker of the MTOR and MEK/ERK pathways[4]. Curcumin’s anti-carcinogenic effects have been linked to inhibition of the MEK/ERK signalling pathway...
in breast carcinogenesis, and researchers continue to explore these potential biomarkers in other cancers. However, ERKs activity in cutaneous malignancy is not well defined in the literature. Hence, study was done to determine if topical curcumin was as efficacious as oral curcumin in a SCC skin xenograft model and elucidate the pathways downregulated by curcumin as potential biomarkers for future chemopreventive studies with the topical curcumin cream. 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). 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 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 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, and have received much attention of researchers. 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. \[11\] 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. \[12\] In this quest, the present work was carried out to monitor the effect of commonly used essential oils namely geraniol, linalool and nerol on skin permeation of curcumin and to elucidate the mechanism of skin permeation enhancement. DSC studies help provide the information about their thermotrop behavior. \[13\] Antiproliferative effects of curcumin were demonstrated in an aggressive skin cancer cell line SRB12-p9 (compared to control). Topical formulation was as effective as oral curcumin at suppressing tumor growth in a mouse skin cancer model. Curcumin at 15 mg administered by oral, topical, or combined formulation significantly reduced tumor growth compared to control. \[14\]

2. Materials and methods

2.1. Materials
Curcumin, geraniol, linanool and nerol, NaOH, NaCl, ethanol, Sodium azide, Potassium dihydrogen orthophosphate, Sodium bromide, Potassium sulfate, HPLC-grade acetonitrile and methanol, tryspin were purchased from reliable sources.

2.3 Preparation of TDDS
5% Transdermal patch of curcumin using 0.5% each of geraniol, nerol and linalool as penetration enhancers, and without any penetration enhancers, in PEG were prepared.

2.4 Preparation of rat skin and in vitro skin permeation studies
Goat skin was taken, and it was cleaned and washed with phosphate buffer of pH 7.4 and prepared for the study. Diffusion studies were performed using Diffusion cell. The effective diffusion area between two half cells was 5.26 cm² 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. The in vitro permeation study was when Donor solution contains penetration enhancer in the vehicle. 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 UV method.
Standard calibration curve of standard curcumin was made at 421 nm. The stock solution of curcumin of 10µg/ml was made in methanol and dilutions from 1-7µg/ml were made.[19]

2.5 Permeation enhancement mechanistic studies

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/cm²) and time in (h). The permeability coefficient (kp) 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 kp in presence of enhancer by kp without enhancer (control).

Therapeutic transdermal daily dose (Td) of curcumin was calculated by the following equation.

\[ T_d = D_o \times F / 100 \]

Where, F is the bioavailability in percentage after oral administration (33% for curcumin) and Do is the oral dose (25 mg for curcumin). The permeated daily dose (Dss) was calculated from the following equation.

\[ D_{ss} = J \times T_a \times t \]

Where, J is the curcumin flux in µg/cm²/h, Ta is the area of diffusion and t is the duration of treatment.

3. Results and discussion

3.1. Skin permeation studies

The increase in Flux is found to be significantly higher when geraniol is used as penetration enhancer. This increase in Flux suggests that a much higher percentage of curcumin can be absorbed with geraniol as enhancer in comparison with the control group, per day.

Table No. 1: Skin Permeation Parameters of In vitro Skin Permeation Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Flux</th>
<th>Enhancement Ratio</th>
<th>% increase in flux</th>
<th>Lag time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>143±1.5</td>
<td>1</td>
<td>-</td>
<td>3.6±0.001</td>
</tr>
<tr>
<td>Test 1</td>
<td>260±1.4</td>
<td>1.6</td>
<td>42</td>
<td>0.04±0.002</td>
</tr>
<tr>
<td>Test 2</td>
<td>222±1.3</td>
<td>1.4</td>
<td>35</td>
<td>0.5±0.002</td>
</tr>
<tr>
<td>Test 3</td>
<td>180±1.2</td>
<td>1.5</td>
<td>37</td>
<td>0.4±0.001</td>
</tr>
</tbody>
</table>

The results of the above two studies suggested the effectiveness of investigated essential oils as penetration enhancer in the following order: geraniol > linalool > nerol.

4. Conclusion

Findings of the above study demonstrate that it is possible to achieve enhanced curcumin flux by test essential oils geraniol, linalool and nerol. However, only geraniolincorporation provided the target flux required to deliver therapeutic dose of curcuminecutaneously. It is concluded that it is feasible to deliver therapeutically effective dose of curcumin via transdermal route using geraniol as penetration enhancer.

5. References