Anti-proliferative effects of an herbal formulated cream on human keratinocytes and its implication for psoriasis treatment

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Abstract: Psoriasis is a chronic inflammatory skin disorder which affects more than 3% of the population worldwide and is characterized histopathologically by proliferative imbalance and abnormal differentiation of epidermal keratinocytes and inflammatory infiltration. Hence, loss of regulation in keratinocyte proliferation and differentiation makes it a typical pathophysiological phenomenon in psoriasis manifestation. Traditionally, herbal products used in treating psoriasis have shown promising effects in several clinical studies with relatively fewer adverse effects, higher remission and lower recurrence rates. In our previous study, the polyherbal formulation of InnoVision’s test material was found to induce AQP-3 expression in keratinocyte cell line. In the present study, we screened the study material for its anti-proliferative properties using cultured human HaCaT keratinocyte cell model. Our experimental results suggest that InnoVision’s Psoriderm Cream is a promising source which can be effectively used as an herb-based topical agent for psoriasis treatment. Evidence is provided that inhibition of keratinocyte proliferation and improving skin hydration via induction of aquaporin-3 stimulation is the possible underlying mechanism for the observed anti-psoriatic action of study material.

Key words: Psoriasis; Human keratinocyte; HaCaT; Anti-proliferation; InnoviSion’s Psoriderm

Introduction

Psoriasis is a benign and chronic hyper-proliferative skin disorder that is conventionally characterized by sudden, periodic appearance of distinctly defined skin conditions ushered mainly by red/thick skin patches with inflamed lesions showing a range of other symptoms such as, dryness, itchiness, blisters/rashes, scales/plaques etc. Amongst many different systemic alterations leading toward the pathogenesis of psoriasis, the preliminary disease initiating activity occurs in the epidermal region of the skin where keratinocytic cells hyper-proliferate, mature and aberrantly differentiate, which is believed to be due to abnormal functioning of the immune system [1-3]. In the process of normal skin growth and development, keratinocyte proliferation occurs in the basal epidermal region and the immature keratinocytes tend to become more mature and differentiated as they move up toward the outer epidermal regions, after which they are unobtrusively shed and rubbed off relatively over a period of time. However, in cases of psoriasis, dysregulation of keratinocytic proliferation and differentiation results in hyperproliferation of keratinocytes accompanied by abnormal differentiation thereby rendering the skin unable to timely shed off these rapidly multiplying and migrating keratinocyte cells, and thus accumulation of these cells on skin results in characteristic psoriatic skin [4-9].

While there is no complete cure for psoriasis, given the intrinsic hyper proliferative nature of epidermal keratinocyte cells during psoriatic conditions, anti-psoriatic therapies aimed at regulation of keratinocytic proliferation and differentiation are highly reliable in treating psoriatic skin, since restoration of homeostatic regulation of keratinocyte proliferation, growth and differentiation is a critical event necessary for normalization and recovery of psoriatic skin [10-12]. Several of the available and established anti-psoriatic treatments are topical therapies such as those involving vitamin-A and D3 analogs, tazarotene dithranol, monomethylfumarate, fumaderm, artemether hydroxyl urea, methotrexate etc., all of which are based on the anti-proliferative and apoptosis inducing properties of the synthetic/semi-synthetic drugs [13-16]. Despite the availability of many of these drug regimens for treating psoriasis, yet none of these can address psoriasis comprehensively due to differential efficacy, disease relapse and the associated side effects posed by them. Thus a great demand exists as part of active pharmacological research to identify newer, alternate treatment modules that can modulate psoriatic conditions by overcoming the drawbacks and limitations of currently available drug therapies, and henceforth offer better cure without disease recurrence and any significant adverse effects with wide range of efficacy, tolerance and safety.

The approach of using natural products for their disease preventive/curative potentials has been traditionally employed in the field of alternate and...
complementary medicine which can be dated back to time immemorial [17, 18]. Plants and herbal based formulas based on individually optimized plant extracts or combined formulations have been reported to be widely implicated in combating various dermatological problems including psoriasis. Studies pertaining to different traditional medicine forms have revealed the potent efficacy of a good number of herbs and formulations in treating psoriasis by exerting their therapeutic action mainly due to anti-proliferative abilities, when tested in keratinocyte cell models. [19-23]. Such studies on the anti-proliferative and apoptotic properties of herbal ingredients and the fact that these mechanisms of alleviating cellular proliferation contribute toward effective anti-psoriatic features provided us an inclination to carry out this study. The goal of the present study aimed to investigate the anti-psoriatic action of a standardized herbal formulation containing optimized extracts for its anti-proliferative activities on HACAT keratinocyte cell model with the purpose of providing scientific evidence for its pharmacological uses.

Materials and Methods

Test system: HaCaT (Human, Keratinocyte)

Maintenance: Stock cells of HaCaT were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/mL) and amphotericin B (5 μg/mL) in an humidified atmosphere of 5% CO2 at 37°C until confluent. The confluent monolayer cultures (HaCaT) was dissociated using TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) and cells were sub-cultured by centrifugation. The stock cultures will be grown in 25cm2 culture flasks.

Preparation of culture for experiment: The confluent monolayer cultures were seeded at 1 x 10⁶ cells/ml into the microtitre plate 96 well (Tarsons, India). The cultures were incubated in humidified atmosphere of 5% CO2 at 37°C until confluent.

Test Substance: Psoriasis cream lotion

Each gram of the study material, Psoriderm contains extracts of: Black mustard (Brassica nigra Linn.), Bakuchi (Pseudotsuga coryliforma Linn.), Neem (Azadirachta indica Linn.), Manjistha (Rubia cordifolia Linn.), Gotu Kola (Centella asiatica Linn.), Licorice (Glycyrrhiza glabra Linn.), Coconut palm (Cocos nucifera Linn.).

Preparation of test doses

For studies, each test substance was weighed and mixed to obtain the desired concentration and dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/mL concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out studies.

Determination of Cytotoxicity and in HaCaT cell line

The monolayer cell culture was trypsinized and the cell count was adjusted to 60,000 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μL of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37o C in 5% CO2 atmosphere. After 24 h, the drug solutions in the wells were discarded and 50 μL of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 48 h. MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO2 atmosphere. The supernatant was removed and 100 μL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

Anti-proliferative activity of test substance in HaCaT cell line

The proliferative potential of test substances was analyzed as a function of protein determination by Bradford method. Briefly the cells were treated with test substance similar to the cytotoxicity in microtitre well at different concentration and incubated for 24 hour at 37°C in 5% CO2 atmosphere. After incubation, the cell supernatants were discarded and the protein content was determined by lysing the cells by 1N NaOH treatment.

Results

Psoriasis cream Lotion showed low cytotoxicity with CTC50 value over 1000 μg/mL. Test substance exhibited moderate anti-proliferative property in a dose dependent manner over control.

Table 1: Cytotoxic properties of test substances against HaCaT cell line

<table>
<thead>
<tr>
<th>Name of sample</th>
<th>Test conc. (%)</th>
<th>Cytotoxicity (%)</th>
<th>CTC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis cream lotion</td>
<td>1000</td>
<td>44.51 ± 2.1</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>3.03 ± 3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>23.27 ± 3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>13.73 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>670 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>
Epidermal homeostasis - in simpler terms could be understood as the restoration of normal epidermal tissue structure of the skin by maintaining a fine delicate balance between proliferation and differentiation of the most prominent skin cell type, keratinocytes, in the basal layer of the epidermis and the apoptotic process of keratinocytic cell death in the superficial layer of the epidermis [24-28]. In view of this, proliferative imbalance with respect to keratinocytes in the epidermis and defects in the subsequent apoptotic processes which lead to epidermal disintegration and hyperplasia are strongly implicated in the pathogenesis of psoriasis [27-29]. Agents that are able to inhibit keratinocyte proliferation/differentiation and induce keratinocyte apoptosis therefore possess good potential for being employed as effective agents in psoriasis treatment. Most of the pharmacologically available anti-psoriatic treatments deliver their therapeutic effects mainly by targeting the hyper-proliferative activity of keratinocytes [9, 11, 36-38]. Thus, an effective and reliable anti-psoriatic medication could be thought of as one that ideally displays low toxicity and maintains epidermal homeostasis by inhibiting keratinocyte hyperproliferation and/or abnormal differentiation with skin rejuvenating effects. In relation to it, alternate, natural plant/herb based anti-psoriatic therapies have been more popular these days owing to their safety and lack of side effects [32-34].

The present investigation relates to the evaluation of a polyherbal formulation designed to address the problem of psoriasis mainly by reducing abnormal hyper-proliferation of keratinocytes and improving skin moisturization. Data indicate the potent suppressive action of the study material on cultured HaCaT keratinocyte cells. Previous study related to the same study material indicated the material’s hydrating/moisturising effects by virtue of its aquaporin-3 stimulatory properties (aquaporin-3 (AQP-3) is a keratinocytic membrane-bound water channelizing protein). Altered skin barrier function in psoriatic skin can result in transepidermal loss of water [39]. A substantiating number of other reports have suggested that moisturizing agents/creams can limit transepidermal water loss and can contribute toward attenuation of epidermal keratinocytic hyper-proliferation and induce differentiation in conditions of psoriasis [36-38]. Similar observations have been made when taken together the findings of our present and previous studies concerning the anti-psoriatic effects of the study material. These results must indicate the anti-proliferative and moisturizing/hydrating (via AQP-3 up-regulation) as the probable mechanism of action of the study material, InnoVision’s Psoriderm Cream, in addressing psoriatic skin. To add to it, several classes of biological compounds present in the bioactive herbal composition of the study material, by possessing a wide range of activities such as, antioxidant, immunosuppressive, haemostatic, white blood cell count enhancing and anticancer properties, might have synergistically been responsible for the observed anti-proliferative effects of the test material on keratinocyte proliferation.

**Conclusion**

Collectively, the results of this study confirm that the study material, InnoVision’s Psoriderm Cream, is capable of inhibiting mitotic proliferation rate in cultured HaCaT keratinocytes. Given the targeting of keratinocyte as a relevant biological target, the

**Table 2: Anti-proliferative activity of test drugs against HaCaT cell line**

<table>
<thead>
<tr>
<th>Name of sample</th>
<th>Test conc. (%)</th>
<th>% Anti-proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis cream</td>
<td>250</td>
<td>16.32 ± 3.2</td>
</tr>
<tr>
<td>Psoriasis cream</td>
<td>125</td>
<td>9.01 ± 1.0</td>
</tr>
</tbody>
</table>

**Table 3: Protein conc. of the test substance against HaCaT cell line**

<table>
<thead>
<tr>
<th>Name of sample</th>
<th>Test conc. (µg/mL)</th>
<th>Protein conc. (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.991</td>
</tr>
<tr>
<td>Psoriasis cream</td>
<td>250</td>
<td>0.730</td>
</tr>
<tr>
<td>Psoriasis cream</td>
<td>125</td>
<td>0.801</td>
</tr>
</tbody>
</table>

**Figure 1: Cytotoxicity and overall inference of test substance**

**Discussion**

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observed anti-proliferative actions might probably provide the underlying mode of action of the test substance and also renders it as a promising therapeutic herb based candidate for treating psoriasis.

References


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