EVALUATION OF TOLENORM OIL AND TOLENORM OINTMENT IN ‘NEAR SKIN’ MODEL FOR THE TREATMENT OF VITILIGO

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Abstract

Combination of Tolenorm oil and Tolenorm ointment was tested on co-culture of melanocytes and keratinocytes in vitro to understand whether the effect of these Siddha drugs results in the transfer of melanin from melanocytes to keratinocytes. The findings of the present study authenticates the established likely mechanism of action of these Siddha drugs with reference to up-regulation of tyrosinase activity, melanogenesis, dendrite formation and increase in dendrite length directly impact the pigment transfer.

Introduction

Tolenorm oil and Tolenorm ointment are the proprietary Siddha medicines of Dr.JRK’s Siddha Research and Pharmaceuticals. Extensive research work has been done on both these Siddha drugs and has established the likely mechanism of action of these drugs at melanocyte level [1, 2].

Findings of our earlier cell culture studies have clearly shown that these siddha drugs may persuade the melanocytes to produce tyrosinase enzyme and the process of melanogenesis [3]. Further the previous studies also have shown the role of the above drugs in influencing the dendrite formation in melanocytes. The herbal ingredients used in the formulation of Tolenorm oil and Tolenorm ointment have great Siddha legacy [4]. Further, these drugs also have proven usefulness in the treatment of Vitiligo ever since the drugs were introduced in the market.

All the above cell culture-based studies have undoubtedly established the clinical efficacy and the likely mechanism of action of these drugs. However, all the above cell culture studies were done at the level of melanocytes.

The mechanism of pigmentogenesis in mammalian system is quite complex [5]. It is not just the melanocytes are responsible for the skin colour, but the ability of keratinocytes to receive the pigment effectively also plays a significant role in the skin colour [6]. Any quantitative or qualitative defects in the melanocytes do affect in the melanosome transfer to keratinocytes and that can result in pigmentary disorders [7].

Many AYUSH preparations are available in the market for the treatment of Vitiligo, however the real efficacy of all such products remain mystery. For the effective treatment of Vitiligo, the drug must act both at melanocytes and also in the transfer of melanosomes to keratinocytes.

In the present study, the efficacy of Tolenorm oil and Tolenorm ointment has been scientifically established through the use of co-culture of melanocytes and keratinocytes at the ratio at which they are present in the skin (near skin model) [8, 9 & 10].

The biochemical responses like up-regulation of tyrosinase activity, melanogenesis and increase in the number and length of dendrites due to the Siddha drugs whether also influencing in the transfer of melanin to keratinocyte can be established systematically through ‘near skin model’ (co-culture method). Findings of the study are discussed in the paper.
Materials and methods

Co-culture and treatment

B16F10 melanocytes and HaCaT cell keratinocytes were used for the co-culture experiment. HaCaT cells at 2.5 x 10^5 per well in six-well plates were maintained in culture medium [8]. After two days, co-cultures of melanocytes and keratinocytes were made in culture media (keratinocyte media + melanocyte media) with the initial seeding ratio of keratinocytes to melanocytes being 5:1. After one day of co-culture, the combination of Tolenorm oil and Tolenorm ointment at 40µg/ml (equal proportion) were prepared separately in double distilled water containing 5% glycerin and added into the co-culture medium and then were incubated for 48 hours. After 48 hours of incubation, the cells were stained by Fontana – Masson stain [11] and were examined under microscope to evaluate the melanin uptake by the keratinocytes as well as protrusion and elongation of dendrites.

Result

On microscopic examination, it was observed that nearly 40% of keratinocytes had received melanin. Further, the dendrite protrusions from melanocytes leaning towards keratinocytes were also observed [Fig-1].

![Fig-1 Co-culture experiment demonstrating dendrite formation and uptake of melanin by keratinocytes treated with combination of Tolenorm oil and Tolenorm ointment.](image-url)
Discussion
Findings of the ‘near skin model’ study have re-established the pre-existing clinical proof of Tolenorm oil and Tolenorm ointment for the treatment of Vitiligo. The clinical proof was gathered over the years from large number of medical practitioners of Siddha, Ayurveda and Dermatology in different parts of India. Our previous studies have clearly shown that both Tolenorm oil and Tolenorm ointment were effective in stimulating B16F10 melanocytes to produce tyrosinase enzyme and the process of melanogenesis. Further, these Siddha drugs are also found to increase the number and length of dendrites in melanocytes [12].

One of the active constituents of *Piper nigrum* that present in Tolenorm oil has sufficient scientific proof for up-regulating melanogenesis [13]. In our previous study, we have observed that Tolenorm oil and Tolenorm ointment up-regulates melanogenesis both at pre- and post-transcriptional levels [14].

Findings of the present study have clearly shown that the combination of Tolenorm oil and Tolenorm ointment has resulted in the effective transfer of melanin to keratinocytes as well.

The real cause of Vitiligo is idiopathic although a few predisposing factors were found to correlate with the disease. One of the fundamental prerequisites for the treatment success is the number and quality of functional melanocytes at the site or nearby the depigmented region.

Interestingly, several patients with functioning melanocytes were found to suffer from Vitiligo. The auto-immune scavenging of the melanocytes is hypothesized for the above cause. The poor/ineffective transfer of the synthesized melanin from melanosomes to keratinocytes is considered to be yet another reason for the above. There are several biochemical and anatomical mechanisms are involved in melanin transfer. The formation of large number of dendrites and their length play a major role in the melanin transfer.

The combination of Tolenorm oil and Tolenorm ointment seems to trigger cascading effect such as

1. Tyrosinase production
2. Melanin synthesis
3. Increase in the length and formation of dendrites in melanocytes
4. Transfer of melanin to keratinocytes

The question of why the combination of Tolenorm oil and Tolenorm ointment could not revolutionize the treatment of Vitiligo lies more in the pathogenesis of Vitiligo than its therapeutics. All Vitiligo patients are not responding evenly and equally to Tolenorm oil and Tolenorm ointment, whereas some are responding quickly and effectively. We presume that the etiology of Vitiligo in all those who responds faster to Tolenorm oil and Tolenorm ointment may likely to fall short of one or all of the above four defects. Perhaps, the etiology of Vitiligo in all those patients who had responded poorly to Tolenorm oil and Tolenorm ointment might have fallen outside the premise of the most known causes of Vitiligo.

A proper diagnosis for the real cause of Vitiligo is not available till date. Therefore, the patients are more often go hopeless or get lost in the world of several products that come to the market with tall promises.

The co-culture findings highlight not just the science or efficacy of Tolenorm oil and Tolenorm ointment but indeed about the great science of Siddha system of medicine. Above drugs are formulated fully in line with Siddha principles using Siddha herbs. The study findings come not only as an assurance for the treatment of Vitiligo but also validate great science of Siddha medicine of system.
References