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Surrogate Skin-Gelatin-Protein Complex based evaluation of Psorolin B and Psoriasis management

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ABSTRACT

For the rapid evaluation of any anti-psoriatic preparations require a credible and scientifically acceptable model that should at least partially resemble the skin. The skin is composed by cholesterol, proteins like elastin, keratin etc. Therefore, a semi-permeable membrane composed on most of such agents may help us to evaluate how the absorption and associated permeation of the drug happens through such membrane. We have developed a surrogate skin model using the essential chemical composition of the skin and have evaluated the absorption kinetics of Psorolin B and details are presented in the article.

Keywords: Psorolin B, lipo-hydro balance, xerotic skin, psoriasis

INTRODUCTION

Anti-psoriatic preparations require predictable in vitro test models so that the clinical efficacy of the formulation can be ascertained objectively and also with reasonable conformity. Pathology of Psoriasis is quite imbricate and complex to comprehend and elucidate, therefore the topical preparations for Psoriasis in general are bound to exhibit varied therapeutic benefits due to the above pathological reason.[1, 2, 3] Even the steroidal preparations may show poor activity when the inflammatory trigger on uphill and such medical situation often warrants oral or parental mitotic inhibitors like methotrexate.[4' 5' 6]

The topical preparations for Psoriasis required to exhibit not just the desired therapeutic benefit alone but also must address the organoleptic demands of the Psoriatic skin as Psoriasis in untreatable, chronic disease with the pathological cycle often repeats over time. The organoleptic demands once addressed, immediately the skin texture and barrier benefit improves which lay foundation to better prognosis by the subsequent treatment products. Use of such products, further would assure prolonged symptom free skin.

Rapid evaluation of topical anti Psoriatic preparations suffers largely due to the lack of simple, rapid, cost effective method that would help us to evaluate the organoleptic benefit of the preparation. The in vitro model must reflect the skin to 'some' extent at least at 'physical' level, only then the desired benefit prediction and extrapolation can be made, objectively.

In the present research article, we have developed gelatin-protein skin equivalent membrane model and tested a proprietary Siddha drug - Psorolin B for its organoleptic benefit possibly at the functional level. Based on our experiment, we have made a postulate on the possible therapeutic effect of Psorolin B and the likely treatment outcome in Psoriatic patients treated with Psorolin alone or in combination with other conventional drugs. Details of the research findings are presented in the paper.

MATERIALS AND METHODS

Gelatin, elastin, bovine serum albumin (BSA), double distilled water and cholesterol of fine/pure quality were used for the experiment.

In brief, 1.7% gelatin solution was prepared in warm double distilled water and then 0.02% of BSA, 0.01% elastin and 0.1% cholesterol were incorporated and gently stirred to achieve complete miscibility of all constituents. After adjusting the pH to 7.1, the molten material was spread over a glass plate and with the help of a fine roller; the material was spread into a very thin membrane over the glass plate. Even thickness of the membrane was achieved using uniform pressure and speed of spreading.

Sufficient number of such membrane plates were made and allowed to dry for 24 hr for the experiment.

Physical attributes of the membrane versus time

The membrane plates were stored at two different temperature zones such as RT and 42°C with relative humidity of 45 and 80%. The wind movement was regulated, where the inward movement of fresh air or the outward movement of air from the chamber was controlled.

The membrane shrinkage, peripheral detachment, central separation, wrinkle formation, spider net like crack formation, colour change, opacification vis-à-vis time were recorded. The above physical attributes of the control membrane were used as reference to compare how Psorolin B intervention has influenced the above physical attributes in treatment group.

0.1% of Psorolin B was incorporated into the gelatin slurry while membrane preparation and the difference between Psorolin B topically applied group versus Psorolin B incorporated group were recorded and compared with control.

Dissection Microscope was used to observe and record all finer details of the membrane wherever required.

RESULTS

In control group, the surrogate skin exhibited almost all characteristics of aging due to moisture loss such as peripheral detachment, shrinkage, central separation, wrinkle formation, spider net like cracking by 10^{th} day. The above change was more pronounced when the model was incubated at elevated temperature zone with low humidity rate, Table 1 – 4.

When the surrogate skin was topically treated with Psorolin B 2mg/cm^2, exhibited lesser degree of physical change both in ambient and elevated temperature zones with low humidity, Table 5-8.

When Psorolin B was incorporated into the surrogate skin model, the degree of change on day 10 was least irrespective the temperature and humidity of the chamber where the surrogate skin was incubated, Table 9-12

Table 1 Changes in physical attributes of control skin equivalent membrane stored at 26°C/45%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	++	+++	+++	+++
Shrinkage	-	++	+++	+++	+++
Central separation	-	-	+	++	+++
Wrinkle formation	-	++	+++	+++	+++
Spider net like crack	-	++	+++	+++	+++
Colour change/opacification	-	-	-	-	+

Table 2 Changes in physical attributes of control skin equivalent membrane stored at 26°C/80%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	+	+	++	++
Shrinkage	-	++	++	++	++
Central separation	-	-	-	+	++
Wrinkle formation	-	++	++	++	++
Spider net like crack	-	+	+	++	++
Colour change/opacification	-	-	-	-	-

Table 3 Changes in physical attributes of control skin equivalent membrane stored at 42°C/45%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	+++	+++	+++	+++
Shrinkage	-	+++	+++	+++	+++
Central separation	-	+	+	++	+++
Wrinkle formation	-	+++	+++	+++	+++
Spider net like crack	-	+++	+++	+++	+++
Colour change/opacification	-	-	-	+	+

Table 4 Changes in physical attributes of control skin equivalent membrane stored at 42°C/80%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	++	+++	+++	+++
Shrinkage	-	+++	+++	+++	+++
Central separation	-	+	+	++	+++
Wrinkle formation	-	++	++	+++	+++
Spider net like crack	-	++	+++	+++	+++
Colour change/opacification	-	-	-	+	+

Table 5 Changes in physical attributes of Psorolin B topically treated skin equivalent membrane stored at 26°C/45%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	-
Shrinkage	-	-	-	-	+
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	+
Spider net like crack	-	-	-	-	-
Colour change/opacification	-	-	-	-	-

Table 6 Changes in physical attributes of Psorolin B topically treated skin equivalent membrane stored at 26°C/80%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	+
Shrinkage	-	-	-	-	+
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	-
Spider net like crack	-	-	-		-
Colour change/opacification	-	-	-	-	-

Table 7 Changes in physical attributes of Psorolin B topically treated skin equivalent membrane stored at 42°C/45%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	+
Shrinkage	-	-	-	-	+
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	+
Spider net like crack	-	-	-	-	-
Colour change/opacification	-	-	-	-	-

Table 8 Changes in physical attributes of Psorolin B topically treated skin equivalent membrane stored at 42°C/80%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	+
Shrinkage	-	-	-	-	+
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	-
Spider net like crack	-	-	-		-
Colour change/opacification	-	-	-	-	-

Table 9 Changes in physical attributes of Psorolin B incorporated skin equivalent membrane stored at 26°C/45%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	-
Shrinkage	-	-	-	-	-
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	-
Spider net like crack	-	-	-	-	-
Colour change/opacification	-	-	-	-	-

Table 10 Changes in physical attributes of Psorolin B incorporated skin equivalent membrane stored at 26°C/80%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	-
Shrinkage	-	-	-	-	-
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	-
Spider net like crack	-	-	-		-
Colour change/opacification	-	-	-	-	-

Table 11 Changes in physical attributes of Psorolin B incorporated skin equivalent membrane stored at 42°C/45%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	+
Shrinkage	-	-	-	-	+
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	+
Spider net like crack	-	-	-	-	-
Colour change/opacification	-	-	-	-	-

Table 12 Changes in physical attributes of Psorolin B incorporated skin equivalent membrane stored at 42°C/80%

Attributes	Obvious change/time in days				
	2	4	6	8	10
Peripheral detachment	-	-	-	-	+
Shrinkage	-	-	-	-	+
Central separation	-	-	-	-	-
Wrinkle formation	-	-	-	-	-
Spider net like crack	-	-	-		-
Colour change/opacification	-	-	-	-	-

DISCUSSION

The gelatin-protein complex surrogate skin constructed by us has allowed us to predict the organoleptic benefit of Psorolin B ointment greatly as the model developed by us is simple, cost effective and less time consuming to evaluate and finally very sensitive. Most nearest biochemical characteristics of skin we could evoke through our new construct by meeting all essential requirements of the skin such as elastin, protein moiety, cholesterol and moisture through gelatin entrapment method. Further the membrane is semipermeable; however the selective permeability of the same is not evaluated.

Our evaluation based on untreated control showed that Psorolin B treatment indeed has increased the longevity of the surrogate skin as most of the physical attributes that we measured did not occur in treatment group than in control. However, Psorolin B incorporated group showed greater protection than the one received topical treatment of Psorolin B suggesting the permeation of the ointment may be

responsible for such difference. All the physical attributes that we studied such as peripheral detachment, shrinkage, central separation, wrinkle formation and spider net like cracking are the common in xerotic skin.

Xerotic skin occurs as a result of loss of the functioning of the skin or due to irregular deposition of stratum corneum cells or accelerated skin turn over cycle.[7] The skin condition, Psoriasis often leads to such situation. Once the lipo-hydro balance is regulated, new cell formation and rejuvenated function can be achieved. Most of the anti-psoriatic topical preparations largely focus on either cell proliferation inhibition or suppressing the inflammatory reaction and beyond that not much organoleptic demand of the skin is addressed.

After understanding most of the nuances of pathology of psoriasis, we have formulated Psorolin B, a proprietary Siddha drug with several medicinal herbs such as Boswellia serrata, Hydocarpus idthyana, Wrightia tinctoria, Cynodon dactylon along with the source of vitamin D, E and red ochre.

Psoriasis being a chronic skin disease with irregularly repeated cycle of pathology requires treatment also to calm the elicitation point which is obviously the organoleptic aspect. The external factors (temperature, load of allergen etc.,) are the common triggers that elicit the clinical phase of psoriasis. When the integrity of the skin is protected (lipo-hydro balance), the trigger of external stimuli can be nullified greatly. Psorolin B is formulated with herbal drugs along with fine fats such as wheat germ oil, cheese (rich source of vitamin D) and vitamin E. Collectively the base ingredients would offer hour long antioxidant effect along with skin repairing benefit and barrier effect. The sustained moisture would enable the cell cycle to revert to normal in psoriatic skin besides the herbal drugs annulling the pro-inflammatory mediators from expression thus resulting in aggrieved clinical phase of psoriasis.

Our present study has not only proved the efficacy of psorolin B but also the usefulness of the method that we have developed.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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