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Modulation S100A7 on KC surface and associated immune deflection: Psorolin B ointment

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Abstract
The present study describe the effect of Psorolin B ointment in modulating the expression of S100A7 protein by HaCaT cell in cell culture method as well as on human volunteer where the inflammatory reaction was initiated with Capsaicin. Psorolin B application post capsaicin treatment has shown remarkable reduction of S100A7 protein not the capsacin induction post Psorolin B application. The findings suggest that Psorolin B may be effective during pre-inflammatory stage not when the inflammatory reaction has touched the peak. Based on the study finding the treatment recommendation for Psoriasis has been evolved and S100A7 is the protein predominantly invite and elicit the inflammatory events during Psoriasis.

Keywords: S100A7 protein, Psorolin B, keratinolytic agent, inflammatory psoriasis

Introduction
The auto-immune disease of the skin- Psoriasis has attracted the attention of scientists across the world to unravel the etiology so that the treatment can be targeted to deal the recurrence if not, the complete cure [1]. But the existence of multiple pathological factors and accompanying co-morbidity conditions has made the understanding of the disease muddling and the treatment options are largely limited to keratinolytic agents, immune suppressants and lavish use of skin hydrating/moisturizing preparations [2]. Further, the specific line of therapy adopted is based on the clinical manifestation of the disease where the use of methotrexate or other similar medicaments are determined by the dermatologist after considering several aspects [3].

S100A7 protein referred as 'psoriasin', is greatly expressed by the cytoplasmic region of keratinocytes and the level of expression of such protein was high during psoriasis and other skin inflammatory conditions [4] such as atopic dermatitis, mycosis fungoides, Darier's disease, and inflammatory lichen sclerosus.

S100A7 protein is believed to function as chemotactic agent over the surface of keratinocytes to attract cytokines, CD4+ lymphocytes and neutrophils. During wound healing where the epithelization is high and so is seen the expression of S100A7 [5], whereas, during normal skin cell cycle turnover time, the expression of same protein was seen low. One of the attributes associated with S100A7 is antimicrobial and immune defense, but it begs, more evidence. In the case of Psoriasis, the expression the protein may have counter effect, inviting larger local immune flare up, worsening the disease.

The expression of S100A7 measurement therefore may offer a perfect understanding of the therapeutic effect of a drug which we intend to screen or evaluate for psoriasis and or other above listed skin conditions. The drug may not have any apparent effect on larger immunology of the skin but may have specific effect in limiting the expression of S100A7 and thereby the drug may offer the much needed relief to psoriasis.

In the present study we have evaluated the effect of Psorolin B as well as the individual herbal ingredients of the formulation in modulating the expression of S100A7 in HaCaT cells subjected to stress as well as in the skin washing in healthy volunteer to understand the therapeutic value of Psorolin B in the treatment of psoriasis. Details are presented in the article.

Materials and Methods

Description of Psorolin Bointment

Psorolin B ointment is a proprietary Siddha drug of Dr JRK's Research and Pharmaceuticals Pvt., Ltd., Chennai. The drug is composed of the following herbal drugs such as Wrightia tinctoria (WT), Boswellia serrata (BS), Cynodon dactylon (CD) and Hydnocarpus ighdiana in an ointment base.

Extracts of the above medicinal herbs was prepared in coconut oil by boiling process where the proportion of solute to solvent was maintained at 1%. The oil thus obtained was evaluated for the activity.

HaCaT cell culture based assay

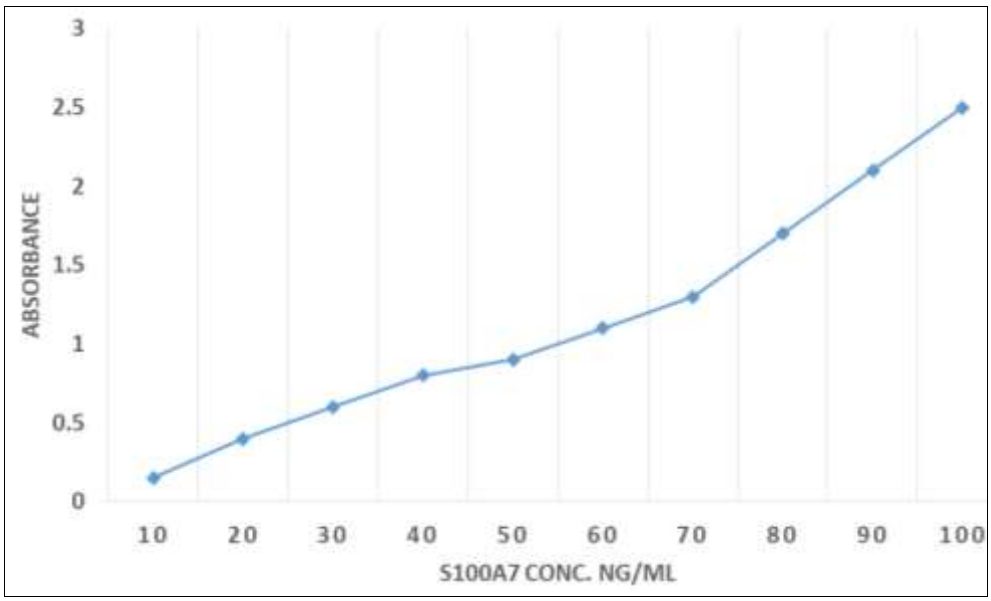
The cells were cultured as per the convention [6] and one group of the cells were treated with the following concentrations (5, 10, 15µg/ml) of either Psorolin B or the individual herbal ingredients into the cell culture medium. 48 hr after treatment, the cell culture medium along with the cells was aspirated, sonicated, centrifuged, made into aliquots and then assayed for S100A7.

Method of assay of S100A7

Sandwich ELISA method was employed for the above purpose [7]. The polyclonal antibody specific against S100A7 pre-coated microplate was used. The skin washings and the cell suspension in the case of HaCaT cell was loaded into the microliter plate and incubated for 1 hr and then the plate was washed to remove the unbound substances. HRP conjugated polyclonal antibody specific for human S100A7 is then added to the wells then the plate was washed to remove all unbound antibody HRP conjugate.

The remaining conjugate was allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction was stopped by addition of acidic solution. Then the plate was read at 450 nm.

A standard curve using S100A7 protein at varying concentration was constructed using absorbance values and the value obtained in the test was compared with the standard plot to understand the quantity of S100A7 in treated and untreated group.



Graph 1: Standard curve of S100A7

Psorolin B treatment and collection of skin washing

Five healthy volunteers in the formulation group would otherwise routinely check the formulations over the skin to understand the organoleptic aspect at the development stage had taken part in the study.

The glabrous skin in the volar forearm region of right and left hand were chosen for the study. In brief, 2 cm^2 area (4 regions) of the skin in one of the arms in each volunteer was pretreated with Psorolin B ointment and after an hour, the region was induced with 0.1 ml of 0.3 µg/ml of capsaicin solution and gently rubbed and left undisturbed. On the other study site, the capsaicin treatment was followed as per the time specified (5 minutes) and then Psorolin B ointment was applied and rested.

Skin washing was collected at different time interval in double distilled and then the skin washing was centrifuged, made into aliquots and then assayed [8].

Result

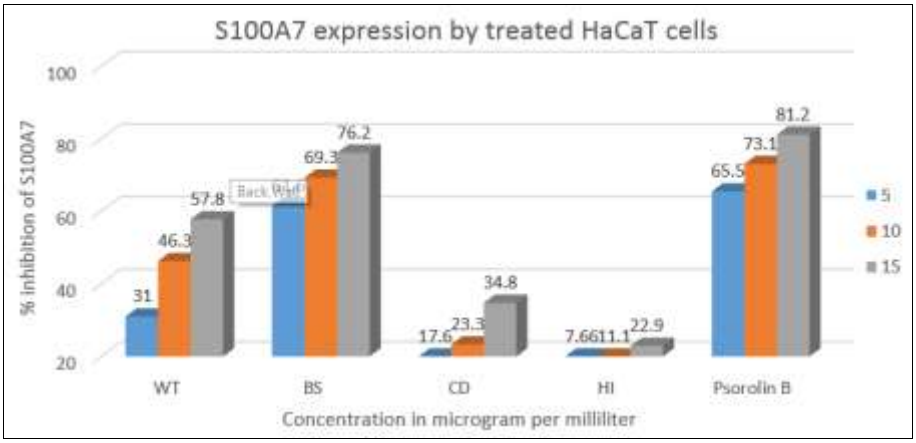
Psorolin B ointment and Boswellia serrata treatment resulted in lowest expression of S100A7 protein by HaCaT cells, followed by Wrightia tinctoria, Cynodon dactylon and Hydnocarpus igdhiana. Table-1.

Table 1: Expression of S100A7 by HaCaT cell

Test detail	Conc. in µg/ml/ Absorbance value		
	5	10	15
Wrightia tinctoria (WT)	1.8	1.4	1.1
Boswellia serrata (BS)	1.0	0.8	0.62
Cynodon dactylon (CD)	2.15	2.0	1.70
Hydnocarpus ighdiana	2.41	2.3	2.01
Psorolin B	0.90	0.70	0.49
Control	2.61		

The graphical representation show the expression of S100A7 by HaCaT cells vis-à-vis treatment. The OD value of the test was calculated with the control using the formula

Percentage inhibition of S100A7= Control- test/control X 100



Graph 2: 1S100A7 expression by treated HaCaT cells

Table 2: Quantification of S100A7 protein from OD value using standard plot

Sample details	Concentration Of S100 A7 (ng/ml) versus conc. of samples		
	5	**10**	**15**
WT	≈ 85	≈ 75	≈ 60
BS	≈ 60	≈ 40	≈ 30
CD	≈ 93	≈ 90	≈ 85
HI	≈ 95	≈ 90	≈ 90
Psorolin B	≈ 50	≈ 40	≈ 25
Control	100%		

A clear directional decrease in S100A7 release by HaCaT cells was observed vis-à-vis the concentration of herbal drugs and Psorolin B tested.

Capsaicin induction one hour after Psorolin B treatment has not affected in the expression of S100A7 when compared to control and so was the effect of individual herbs, Table 3.

Effect of Psorolin B in reducing expression of S100A7 by skin

Table 3: Psorolin B and then Capsaicin

Psorolin B treatment and then Capsaicin treatment	Time interval in minutes/ S100A7 in ng/ml		
	10	**30**	**45**
Wrightia tinctoria (WT)	12	11	14
Boswellia serrata (BS)	11	12	12
Cynodon dactylon (CD)	13	11	12
Hydnocarpus ighdiana	12	13	12
Psorolin B	13	14	13
Negative control	6		
Positive control - Capsaicin	14		

Whereas, Psorolin B treatment after capsaicin induction has significantly reduced the expression of S100A7. Boswellia serrata and Psorolin B ointment had significant effect over Wrightia tinctoria in reducing the expression of S100A7. Other two herbs did not show much effect in reducing the expression of the protein, Table 4.

T**able 4:** Capsaicin and then Psorolin B

Capsaicin treatment and then Psorolin B	Time interval in minutes/S100A7 in ng/ml		
	10	**30**	**45**
Wrightia tinctoria (WT)	8	9	8
Boswellia serrata (BS)	5	3	3
Cynodon dactylon (CD)	11	13	12
Hydnocarpus ighdiana (HI)	12	11	13
Psorolin B	4	3	4
Negative control	6		
Positive control	14		

Discussion

S100A7 protein is expressed largely in the cytoplasm and periphery of the keratinocytes especially during formation of new cells or epithelialization. The protein is considered as marker of inflammation as it is believed that this protein would act as chemotactic agent, attracting cytokines, CD4 cells and neutrophils. Psoriasis is an auto-immune disease where the immune reaction at the level of keratinocyte is elevated which results in immature copying of keratinocytes with no functional benefit. Keratinolytic agents, anti-mutagenic agents [9], immune suppressants are used vastly in the treatment of Psoriasis especially during flare up stage

but such therapy often do cause severe side effects and therefore such therapy is adopted only conservatively and not as default treatment choice.

Use of moisturizers and hydrating preparations in the form of cream, ointment, gel or lotion are also used [10] but such products have limited use where the clinical manifestation is silent and not resilient.

Herbal preparations are often used in the treatment of Psoriasis [11] but once again all such preparations have limitations due to the pleomorphic clinical manifestations and contrasting etiology/triggers.

Psorolin B ointment is a herbal drug formulated with certain select herbs that contains chemical moieties having structural/functional similarity with vitamin D and other required benefits like elastin and collagen up-regulation besides strong anti-oxidant benefit. Earlier we have also established the histidine-decarboxylase inhibition effect [12, 13] of the ointment resulting in offering two-way benefit of reducing the level of histamine and up-keeping the level of histidine which get lowered during Psoriasis.

The clinical experience with Psorolin B ointment has shown remarkable treatment success in most cases, especially during early stage of inflammatory flare up. Which was often concluded to the possible immune suppressing benefit of Boswellia serrata resins. Steroid like immune suppressing benefit we could not establish for the herb, which made us to investigate the possible other mechanism of action or target site. Further, the synergy of the herbal cohort in the ointment also may have such larger benefit preposition which needs to be unraveled.

In the present investigation, we studied the effect of Psorolin B ointment and the individual herbal ingredients in reducing the expression of S100A7 protein over actively forming keratinocytes. We focused this protein because of its chemotactic property for attracting cytokines, CD4 cells and neutrophils to elicit inflammatory flare up cascade to lead an array of clinical manifestations. If selective targeting of this protein could be achieved, much of the immune over outreach can be reduced and thereby the silent phase of Psoriasis can be prolonged, if not, complete cure is achieved.

Our study revealed that Psorolin B ointment effectively reduced the expression of of S100A7 protein as well as the herbal ingredient - Boswellia serrata. We initially studied the above effect using HaCaT cell *in vitro* and established the benefit. In order to understand the above findings better and validate further, we did a trial on healthy human volunteers. We followed both forward and reverse method where capsaicin induction was followed over the skin to initiated the inflammatory flare up locally. The induction with Capsaicin was followed prior to and after Psorolin B application in the pre-selected volar forearm skin. Assay of S100A7 protein was performed in the skin washing by ELISA. Psorolin B ointment application post Capsaicin induction significantly reduced the expression of the protein and not when Capsaicin induction post Psorolin B application. Reason for the above, we hypothesize may be due to the effect of Psorolin B in having effect during the early inflammatory stage by blocking either the accumulation / occupying the cell surface or the very expression of the protein. When the Capsaicin induction followed after Psorolin B application, the post metabolic effect of the ointment might have gone weak and hence could not bring such effect.

The above observation concurs greatly with the clinical experience being shared by several clinicians treating Psoriasis with Psorolin b ointment where the ointment is quite effective in preventing the inflammatory flare up but does not have such strong effect when the inflammatory flare already having touched the summit.

It is difficult to predict or postulate when the silent clinical condition would turn into violent inflammatory reaction and therefore continuous and regular use of Psorolin B ointment may help greatly to prevent the sudden surge of inflammatory flare-up.

Further, the continuous and regular use of Psorolin B ointment also would provide large spectrum of other benefits much needed by the Psoriatic patient. The selective targeting of the chemotactic site of keratinocyte from inviting the immune cells while offering remedial benefit to Psoriasis also would prove beneficial by not acting as steroid, suppressing the entire immunity of the skin and resulting in various other side effects that are associated with prolonged steroid usage.

The new finding strongly support the selective and safe treatment value of Psorolin B for Psoriasis in preventing the inflammatory flare-up.

Conclusion

The study explored the effects of Psorolin B ointment on the expression of the S100A7 protein in HaCaT cells and human volunteers, focusing on the inflammatory response induced by capsaicin. Psorolin B demonstrated a significant reduction in S100A7 protein expression when applied post-capsaicin treatment, suggesting its potential efficacy in the early stages of inflammation rather than during peak inflammatory response.

Key findings include

1. ***In Vitro* Results:** Psorolin B and Boswellia serrata significantly reduced S100A7 expression in HaCaT cells compared to other individual herbal ingredients.
2. **Human Volunteer Study:** Psorolin B was effective in reducing S100A7 protein levels when applied after capsaicin-induced inflammation. However, pre-treatment with Psorolin B did not yield the same reduction in S100A7 expression following capsaicin application.
3. **Mechanism of Action:** The likely mechanism involves Psorolin B's ability to inhibit S100A7 expression during the early inflammatory phase, thereby reducing the chemotactic attraction of immune cells and mitigating the inflammatory response.

These results highlight Psorolin B's potential as a therapeutic agent for psoriasis, particularly in preventing inflammatory flare-ups. Regular use of Psorolin B could maintain lower levels of S100A7, potentially prolonging periods of remission and offering an alternative to conventional treatments that may have severe side effects.

In summary, Psorolin B shows promise as a safe and targeted treatment option for managing psoriasis by selectively reducing the inflammatory protein S100A7, thereby modulating the local immune response without the broad immunosuppressive effects associated with steroids.

Conflict of Interest

Not available.

Financial Support
Not available.

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