International Journal of Research in Dermatology
Amruthavalli GV et al. Int J Res Dermatol. 2022 Jan;8(1):85-89
http://www.ijord.com

DOI: https://dx.doi.org/10.18203/issn.2455-4529.IntJResDermatol20214918

Original Research Article

Psorolin B: a formulation with synchronized, synergistic scooping of botanicals and associated exodus therapeutic benefit to psoriasis

Amruthavalli G. V.*, Aruna V., Gayathri Rajagopal

Dr. JRK's Research and Pharmaceuticals Pvt, Ltd., Kunrathur, Chennai, Tamil Nadu, India

Received: 20 November 2021
Revised: 18 December 2021
Accepted: 20 December 2021

***Correspondence:**
Dr. Amruthavalli G. V.,
E-mail: amruthavalli_gv@jrkresearch.com

ABSTRACT

Background: Psoriasis is an auto-inflammatory disease condition, with the clinical symptoms of scaling, inflammation and itching. This condition is triggered by external and internal factors such as environment, seasons and free radicles etc. Psorolin B is a proprietary Siddha Medicine indicated for the reduction in the psoriatic symptoms and also for inhibiting the various triggers of psoriasis
Methods: In the present paper, the formulation is evaluated for its anti-oxidant effect, anti-glycation effect, enzymatic activity reduction i.e., elastase, collagenase, lipoxygenase and reduction in COX1 and COX 2 by following the standard methods.
Results: In the present paper, the formulation is evaluated for its anti-oxidant effect, anti-glycation effect, enzymatic activity reduction i.e., elastase, collagenase, lipoxygenase and reduction in COX1 and COX 2 by following the standard methods. The study results show that the product is able to reduce- free radicle scavenging activity, advanced glycation end products, elastase, collagenase, lipoxygenase and COX1 and COX 2
Conclusions: The study results prove that this product can control the pro-inflammatory mediators by inhibiting the triggering factors like free radicles and advanced glycation end products. This also play a major role in inhibiting the elastase and collagenase that helps in reducing the cell cycle turn over. Complete details of the study are explained the paper.

Keywords: Psoriasis, Pro-inflammatory mediators, Psorolin B, Triggers of psoriasis

INTRODUCTION

Psorolin B is a proprietary Siddha drug for the treatment of psoriasis. The formulation Psorolin B is made with select herbs such as *Boswellia serrate, Hydnocarpus laurifolia, Cynodon dactylon and Wrightia tinctoria.* Besides the above Kavikal i.e., iron ocher, salicylic acid and source of vitamin D (cheese) are also used in the formulation.

The topical agents (cream/ointment/oil) either must have steroidal property or must have exodus therapeutic effect on several pathological layers of Psoriasis and only then the patients can expect perceivable effect from such medicament. To maintain Psoriasis at pre-inflammatory level which is often referred in common medical parlance as remission phase strictly depends on the effectiveness of the topical agent used for the maintenance therapy.[1] The pre or pro-drug preparation therefore must have global effect on the most fundamental etiological causes that triggers the inflammation reaction; the pro-inflammatory mediators.[2]

On detailed review of various etio-pathological events of Psoriasis, we learned that inhibition of the following enzymatic and or inflammatory mediators such as nitric

oxide, elastase, collagenase, glycation end products, lipoxygenase and cyclooxygenase may dramatically switch off the inflammatory triggers and would result in sustained benign Psoriatic skin or skin with minimal scaling.[3-7]

In allopathic system of medicine, the active pharmaceutical agent used is single molecule, ultra-pure and also is highly target specific. Therefore, broad spectrum, reticulate, web like therapeutic benefit cannot be achieved with such active pharmaceutical agent (s) used in allopathic system of medicine. Further the allopathic pharmaceutical molecules cannot be used either as pre or pro drug.

In phytomedicine, the botanicals or herbal preparations used are largely semi-purified extracts, contains galaxy of phytoactive molecules with co-operative and competitive therapeutic benefits.[8] Further, only post metabolized constituents can exhibit the therapeutic value and therefore accumulation and associated toxicity are the least with botanical preparations (5a).

In the system of AYUSH, predominantly Siddha and Ayurveda, several herbal preparations are indicated to have very strong effect against psoriasis.[9] However, a synchronized effort to achieve the synergistic effect of those herbs to offer great medical relief to Psoriasis has not yet happened so far.

In Psorolin B, we have scooped up the synergistic value of four important medicinal herbs along with Iron ochre, Salicylic acid and source of vitamin D, cheese. Further the formulation is studied in detail for its effect on several enzymatic and inflammatory molecules that are definitely known to worsen Psoriasis.[10-14]

Our findings clearly suggest that the synergistic effect of several herbs scooping into a formulation that we have attempted in the case of Psorolin B can be very effective for Psoriasis. Details are presented in the paper.

METHODS

Inhibition of nitric oxide production[13]

Nitric oxide (NO) is a diatomic free radical produced from L-arginine by constitutive and inducible NO synthase (cNOS and iNOS) in numerous mammalian cells and tissues. Oxidative damage caused by the action of free radicals which in turn may initiate and promote the progression of inflammatory changes in the skin. The mechanism of inflammation lead injury is attributed, in part due to the release of the reactive oxygen species (ROS) from the activated neutrophils and macrophages. ROS propagate inflammation by stimulating the release of mediators such as NO and cytokines.

Macrophages were seeded at the density of 5×10^3 cells per well in a microtiter plate. The plate was incubated for 12 hours at 37°C with 5% CO2. Then, media of each well was then aspirated, and fresh FBS-free DMEM media was replaced.

Different concentrations of the test products were used for testing. After 1 hour of treatment with samples, cells were stimulated with 1 l g/mL of LPS for 24 hours. The presence of nitrite was determined in cell culture media using commercial NO detection kit. After 10-minute incubation, the absorbance was measured at 540 nm using microplate reader. The amount of nitrite in the media was calculated from sodium nitrite ($NaNO_2$) standard curve.

Collagenase assay[13]

Collagenase is important for cell migration and the remodeling of tissues during repair and regeneration. Collagens are the major fibrous component of animal extracellular connective tissue.

The assay employed was based on spectrophotometric method with some modifications. The assay was performed in 50 mmol/L Tricine buffer (pH 7.5 with 400 mmol/L NaCl and 10 mmol/L CaCl2). Collagenase enzyme from Clostridium histolyticum was dissolved in buffer with the initial concentration of 0.8 unit/mL (as per the activity data). The synthetic substrate N-[3-(2-furyl) acryloyl]-Leu- Gly-Pro-Ala (FALGPA) at 2 mmol/L was dissolved in Tricine buffer. Different concentration of the test products was incubated with the enzyme in the buffer and was incubated for 15 minutes. Then, the substrate was added to the above to start the reaction. Water was used as negative control. Absorbance at 335 nm was measured soon after adding the substrate, and the reading was taken continuously for 20 minutes using a microplate reader. Epigallocatechin gallate (EGCG), 250 lmol/L (0.114 mg/mL), was used as a positive control.

Antiglycation assay[13]

Glycation is a non -enzymatic condensation reaction between reducing sugars and amino groups of proteins that undergo rearrangements to stable ketoamines, leading to the formation of advanced glycation end products (AGEs). Antiglycation activity was determined using the bovine serum albumin assay with slight modification. In the experiment, the final reaction volume was 1.0 mL and carried out in 1.5 mL Eppendorf tubes.

Bovine serum albumin 500 lL (1 mg/mL concentration) was incubated with glucose 400 lL (500 mmol/L final concentration) and different concentrations of the products and 100 lL of phosphate buffer saline was used as the sample control and 100 lL Arbutin as the reference standard.

A negative control was carried out at the same time with BSA 500 lL (1 mg/mL concentration), 400 lL phosphate buffer saline and the different concentrations of the

sample (as described above) incubated under the same conditions. The reaction was allowed to proceed at 60°C for 24 hours and was terminated by adding 10 lL of 100% (W/V) trichloroacetic acid (TCA). The TCA-added mixture was kept at 4°C for 10 minutes and then centrifuged for 4 minutes at 13000 rpm. The precipitate was redissolved in alkaline phosphate buffer saline (pH 10) and was quantified for the relative amount of glycated BSA based on fluoresce intensity by fluorescent microplate reader. The excitation and emission wavelengths that used were 370 nm and 440 nm, respectively. Each concentration of the sample was analyzed thrice. Percentage of inhibition was calculated, and the sample concentration required for the 50% of inhibition was calculated.

Elastase inhibition assay[13,14]

Elastases are matrix degrading enzymes involved in the tissue homeostasis and are mainly produced by the epithelial cells in the skin, lungs, and neutrophils, etc. The neutrophil-derived elastases play a major role in the regulation of vascular injury and inflammation, such as ischemia-reperfusion injury. Elastases are available both as membrane-bound and intracellular forms. Intracellular elastases break down the foreign proteins, whereas the extracellular elastases released by neutrophils and mostly bound to the neutrophil plasma membrane assists neutrophil migration to the inflammation sites by degrading various host proteins, such as extracellular matrix proteins. The target organ of elastase is the matrix protein in skin which imparts the structural and functional integrity to it. During the process of chronological aging, the metabolic events such as formation of advanced glycation end products in the skin draw inflammatory infiltrates leading to the formation of wrinkles. Hence, the elastase inhibitors could serve multiple treatment options for various dermatological problems.

Porcine pancreatic elastase was assayed spectrophotometrically using SANA as substrate. The test products were incubated in a mixture of 200 mmol/L Tris buffer (PH 8.0) and 1 unit of enzyme for 15 minutes. The enzyme reaction was initiated by the addition of the substrate, and then, the mixture was further incubated for 15 minutes at 37°C. The absorbance value was read at 410 nm using UV spectrophotometer.

Lipoxygenase inhibition assay[13]

Spectrophotometric assay was performed for the determination of LOX activity. The method was slightly modified to our laboratory condition. In brief, soybean 15-lipoxygenase (15-LOX) was used for the assay.

Inhibition experiments were run by measuring the loss of soybean 15-LOX activity (5 lg) with 0.2 lmol/L linoleic acid (Sigma) as the substrate prepared in solubilized state in 0.2 mol/L borate buffer (pH 9.0). Inhibition studies in the presence of various concentrations of

The test products were studied using spectrophotometer at 234 nm. A known positive control, nordihydroguaiaretic acid (NDGA) was also used.

The inhibitory effect of the test products was expressed as percentage of enzyme activity inhibition. IC50 indicating the concentration required to inhibit 50% LOX activity that was calculated. The values of hydroperoxide content and lipoxygenase activity were calculated from equation:

Specific activity (LOX) =ΔA.V/ ε.l.C

ΔA is the value of absorbance increase per min; V is the volume of incubation mixture, Σ is the extinction coefficient for linoleic acid ($25\times9\ 10^{-3}$ mol/l/cm),

l is the length of the cuvette (1 cm), and c is the concentration of enzyme in mg (0.005)

The values are mean of 3 independent experiments.

COX1 and COX2 assay

The COX-1 and 2 (human ovine) inhibitor Screening assay kit was used for the present study.

In brief the reaction mixture contains 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2) along with 10 µl/ml of the sample.

The principle employed in the assay utilizes the peroxidase component of the COX catalytic domain. The peroxidase activity is then assayed colorimetrically by monitoring the appearance of the oxidized N, N, N, N'-tetramethyl-pphenylenediamine (TMPD) at 590 nm.

Aspirin (acetylsalicylic acid, 1 mM) was used as positive control. The percentage COX inhibition was calculated using following equation:

COX inhibition activity (%) =1−T/C ×100

Where T=Absorbance of the inhibitor well at 590 nm. C=Absorbance of the 100 % initial activity without inhibitor well at 590 nm.

RESULTS

Psorolin B inhibited nitric oxide production and activity was not greatly influenced by concentration (Table 1).

The collagenase inhibition effect of Psorolin was comparable with EGCG although not in concentration level but at activity level. The concentration of Psorolin B did not influence the activity greatly (Table 2).

Psorolin B exhibited elastase inhibition activity which was not concentration dependent (Table 3).

Table 1: Inhibition of nitric oxide production.

Test details	Nitrate production (%)
LPS (75%)	75
LPS +30 mg/ml Psorolin B	28
LPS+ 20 mg/ml Psorolin B	34
LPS+ 10 mg/ml Psorolin B	38
Untreated cells	14

Table 2: Percentage inhibition of collagenase.

Concentration (mg/ml)	Psorolin B
EGCG 250 micromolar/L (38%)	
10	18
20	22
30	26

Table 3: Percentage inhibition of elastase enzyme vs conc. of product.

Concentration (mg/ml)	Inhibition of elastase enzyme (%)
10	21
20	28
30	36

Psorolin B at 60 mg/ml showed activity in inhibiting glycation end product as against arbutin at 1.8 mg/ml (Table 4).

Table 4: Antiglycation assay.

Active concentration	
Arbutin	1.8 mg/ml
Psorolin B	60 mg/ml

Table 5: Lipoxygenase inhibition assay.

IC50 value	
Nordihydroguaiaretic acid (NDGA)	2 µg/ml
Psorolin B	30 mg/ml

Table 6: COX1 and COX2 assay.

Psorolin B (mg/ml)	Inhibition of COX1 (%)	Inhibition of COX2 (%)
10	18	17
20	21	19
30	34	22
40	38	33
Aspirin 10	46.51	49.45
Positive control lipoxygenase inhibitor nordihydro guaiaretic acid (NDGA)	-	-

Psorolin B as low as 30 mg/ml exhibited 50% inhibition of lipoxygenase enzyme activity (Table 5).

Psorolin B showed activity against both COX 1 and COX 2 enzymes irrespective of the concentration and activity was reasonably comparable with aspirin (Table 6).

DISCUSSION

The polyherbal nature of psorolin B formulation might have been the reason for the exodus pharmacological benefit we have observed in the present study against wide spectrum of bio-chemical and inflammatory mediators such as elastase, collagenase, lipoxygenase, COX 1 and COX 2 and finally the glycation end products and nitric oxide induced cellular damage. All the above biochemical and inflammatory molecules play a significant role in the pathology of psoriasis.[6-8]

To maintain psoriatic skin free from the inflammatory elicitation resulting in non-inflammatory phase strictly demand proper dermal care and use of pro or pre palliative medicaments. Highly target specific single molecule based allopathic drugs may not have diasporic therapeutic value to mitigate various pathological events in psoriasis. However, polyherbal preparations can have a radiating therapeutic benefit because the plant preparations are conglomeration of innumerable phyto-actives with co-operative therapeutic value.

We have made a significant step in synchronizing the synergistic phyto-pharmaceuticals of four important medicinal plants such as *Boswellia serrate, Hydnocarpus laurifolia*, *Cynodon dactylon* and *Wrightia tinctoria* besides red ochre and vitamin D source, cheese. The broad-spectrum therapeutic effect of Psorolin B on various pathological events that trigger the ugly clinical side of psoriasis is possible only due to diverse phyto-pharmaceuticals present in the formulation. Psorolin B further has been tested for its effect on various pro-inflammatory mediators such as IL 8, IL alpha 1 and TNF alpha. The collective therapeutic value of Psorolin B clearly points towards the exalted role of psorolin B in maintaining the psoriatic skin free from inflammatory changes.

The effect of Psorolin B on lipoxiginase, COX 1, COX 2 assumes greater importance because the above enzymes certainly prelude some of the early inflammatory events towards inflammatory phase of psoriasis. Similarly, the enzymes elastase and collagenase also play a significant role in worsening psoriasis. The anti-oxidant benefit during psoriasis treatment has a remarkable rewarding which psorolin B offers to a great extent.

The findings of the study clearly suggest that Psorolin B is an effective polyherbal formulation likely to inhibit cellular events, enzyme pathology and lead molecules of inflammation and would collectively offer symptom free phase of psoriasis.

Limitation

The present study is performed in cell line setup using ELISA which is highly reflective of the immunological situation where the findings were purely based on the direct interaction between the cell-line and test drug.

In clinical condition various pharmacological events may influence the drug absorption, distribution, half-life, metabolism and elimination.

Therefore, an elaborate immunology-based evaluation in clinical condition is necessary to conclude the finding doubtlessly.

CONCLUSION

Considering the unequivocal role of various enzymes in the pathogenesis of psoriasis evaluating the response to a drug will certainly reflect the clinical relevance and therapeutic value of psorolin B. our findings poignantly pointing towards the exalted value of psorolin B in the treatment of psoriasis.

ACKNOWLEDGEMENTS

Authors would like to thanks to organisation Dr. JRK's Research and Pharmaceuticals for providing the required facilities for the successful completion of the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Huang YW, Tsai TF. Remission Duration and Long-Term Outcomes in Patients with Moderate-to-Severe Psoriasis Treated by Biologics or Tofacitinib in Controlled Clinical Trials: A 15-Year Single-Center Experience. Dermatol therapy, 2019;9(3):553-69.
2. Reich K. The concept of psoriasis as a systemic inflammation: implications for disease management. J Eur Acad Dermatol Venereol. 2012;26(2):3-11.
3. Lin X, Huang T. 2016, Oxidative stress in psoriasis and potential therapeutic use of antioxidants. Free Radic Res. 2016;50(6):585-95.
4. Wiedow O, Wiese F, Streit V, Kalm C, Chris E. Lesional Elastase Activity in Psoriasis, Contact Dermatitis, and Atopic Dermatitis. J investigative Dermatol. 1992;99(3):306-9.
5. Uva L, Miguel D, Pinheiro C, Antunes J, Cruz D, Ferreira J, Filipe P. Mechanisms of action of topical corticosteroids in psoriasis. Int j endocrinol. 2012;561018.
6. Metzman MD. Topical 5-lipoxygenase inhibitors in the treatment of psoriasis. J Dermatol Treat. 2020;2(3):117-20
7. Zembowicz A, Mastalerz L, Setkowicz M, Radziszewski W, Szczeklik A. Safety of Cyclooxygenase 2 Inhibitors and Increased Leukotriene Synthesis in Chronic Idiopathic Urticaria with Sensitivity to Nonsteroidal Anti-inflammatory Drugs. Arch Dermatol. 139(12):1577-82.
8. Sun S, Wang Y, Wu A. Influence Factors of the Pharmacokinetics of Herbal Resourced Compounds in Clinical Practice. Evidence-Based Complementary Alternative Med. 2019;1983780:16
9. Shenefelt PD. Herbal Treatment for Dermatologic Disorders. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis. 2011;18.
10. Aruna V, Amruthavalli GV, Gayathri R. Psorolin B-A new 'taxon' in the treatment of psoriasis and the science of electrochemical homeostasis. World j pharmac res. 2021;10(8):554-9.
11. Amruthavalli GV, Aruna V, Gayathri R. Botanical warriors to combat pro-inflammation mediators-A way forward for Psoriasis management. Int Invention Scientific J. 2021;05(9):21-4.
12. Importance of thermo, hydro and lipid chemistry of psorolin b and its treatment significance in psoriasis. Eur j pharmac med res. 2021;8:7.
13. Aruna V, Amruthavalli GV, Gayathri R. Use of cosmetic products for treating certain diseases-Know the science. J Cosmet Dermatol. 2019;18:221-5.
14. Scientific and clinical proof for the use of a cell repair cream formulation for Psoriasis. MST International J Pharma Sci Pharma. 2002;1(1):4-12.

Cite this article as: Amruthavalli GV, Aruna V, Rajagopal G. Psorolin B: a formulation with synchronized, synergistic scooping of botanicals and associated exodus therapeutic benefit to psoriasis. Int J Res Dermatol 2022;8:85-9.