EVALUATION OF ANTI-PSORIATIC POTENTIAL OF A NOVEL POLYHERBAL FORMULATION BY MULTIPARAMETRIC ANALYSIS

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INTRODUCTION / ABSTRACT

- Psoriasis is a chronic, inflammatory skin disorder, characterized by uncontrolled hyper-proliferation of keratinocytes in the epidermis, disturbed apoptosis, over-secretion of cytokines and angiogenic factors.
- Anti-psoriatic drugs function by targeting hyperproliferation of keratinocytes, enhancing apoptosis, suppressing inflammation and angiogenesis.
- There is an unmet need for the development of herbal therapies for psoriasis on account of high cost and side effects associated with conventional treatments.
- Traditional herbal medicines have been widely used for the treatment and management of dermatological inflammatory disorders.
- We have developed a novel aqueous mixture (SIRB-001) of 3 Traditional Chinese Medicine (TCM) based herbs namely, *Rheum palmatum* L. (Da Huang), *Rehmannia glutinosa* Libosch (Sheng di huang) *and Lonicera Japonica (*Jin yin hua)(in the ratio 1:1:3).
- SIRB-001 was found to exert highly efficacious effects in psoriasis patients.
- Hence, we investigated the anti-psoriatic mechanism of SIRB-001 by using *in vitro* cell-based systems.

METHODS

- Da huang (roots), Sheng di huang (roots) and Jin yin hua (flowers) were commercially procured and mixed in the ratio of 1:1:3. Herbs were finely powdered, diluted with water, boiled and cooked at 70°C for 1 h. After centrifugation at 5000 rpm for 15 min, the supernatant was used as main stock for experiments. The yield of SIRB-001 extract was 30 mg/ml.
- The anti-proliferative effect of SIRB-001 (1:100 – 1:5 v/v) was assessed using immortalized human keratinocyte cell line; HaCaT as the test model by MTT assay.
- Pro-apoptotic effect of SIRB-001 was examined by flow cytometry and colorimetric methods.
- Inhibitory effect of SIRB-001 (1:10-1:5 v/v) on inflammatory markers; TNF-α, IFN-γ, IL-6, NO and $sPLA_2$ was determined in HaCaT cells against TNF-α stimulated levels by ELISA. Inhibition of IL-17/IL-23 axis was assessed in immune cells; murine splenocytes and human monocytic cell line (THP-1).
- VEGF-downregulation in HaCaT cells was studied for anti-angiogenic potential.
- Pathway mapping was done by kinase profiling using Z'Lyte assays and Topoisomerase-II activity by Kinetoplast DNA Cleavage assay.
- The synergistic anti-proliferative potential was also evaluated with standard anti-psoriatic agents such as Methotrexate (MTX).

HPLC fingerprinting

- HPLC fingerprinting of SIRB-001 revealed the presence of chlorogenic acid (tR=13.98min), Acteoside (tR=24.22 min) and Rhein (tR=53.76 min) as identified by comparisons to the retention times and UV spectra of authentic standards under identical analytical conditions.

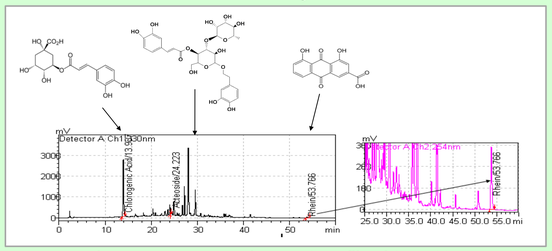


Figure 1 - Representative HPLC profile of SIRB-001

RESULTS

Anti-proliferative effect

- SIRB-001 demonstrated significant (p<0.01) anti-proliferative effect in HaCaT cells. Encouraging results were also observed in combination with MTX.

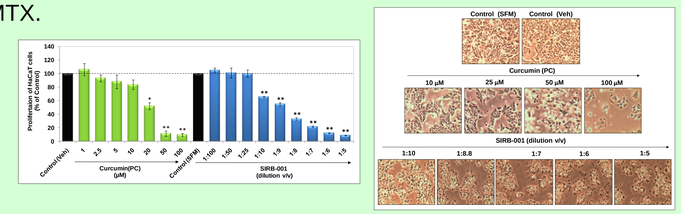


Figure 2 - Inhibition of HaCaT cells proliferation by SIRB-001 after 48 h of treatment

Pro-apoptotic potential

- SIRB-001 exhibited significant (p<0.01) pro-apoptotic effect mediated via early, mid and late markers.

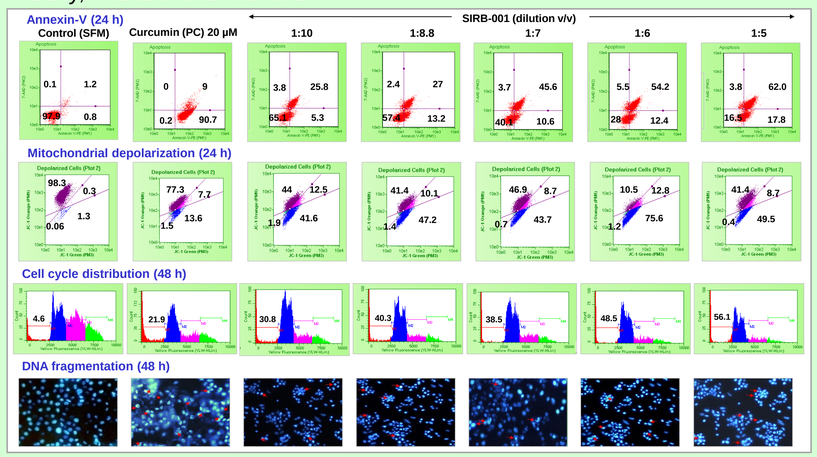


Figure 3 – Apoptotic potential of SIRB-001 in HaCaT cells

Anti-inflammatory activity

- SIRB-001 resulted in significant (p<0.01) downregulation of pro-inflammatory markers (TNF-α, IFN-γ, IL-6, NO, $sPLA_2$) in HaCaT cells and IL-17/IL-23 secretion in immune cells.

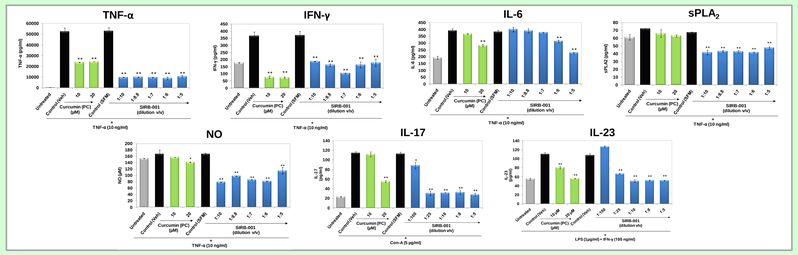


Figure 4 – Inhibitory effect of SIRB-001 on secretion of inflammatory markers after 24 h of treatment

Anti-angiogenic potential

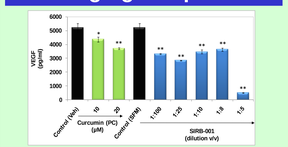
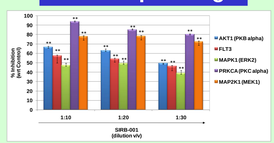


Figure 5 – VEGF inhibition by SIRB-001 in HaCaT cells after 24 h of treatment

Kinase profiling



Topoisomerase-II inhibition

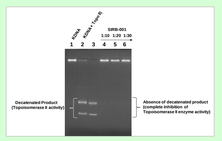
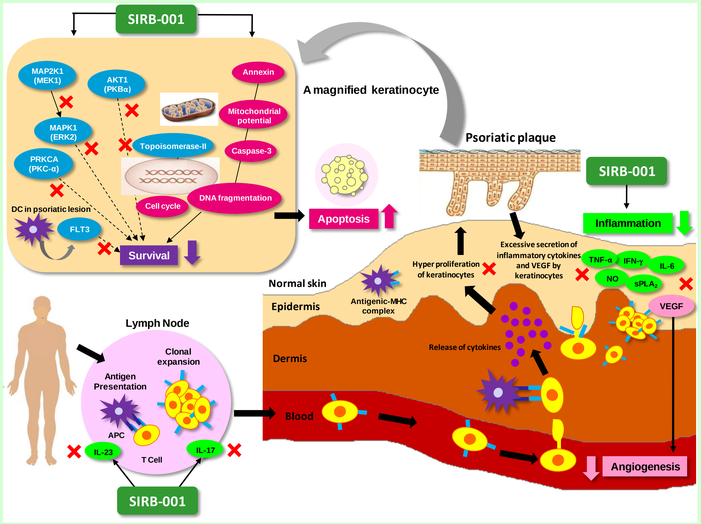


Figure 6 – Inhibitory effect of SIRB-001 on key kinases and Topoisomerase-II activity

PUTATIVE MECHANISM OF ACTION OF SIRB-001



CONCLUSIONS

- SIRB-001 has exhibited *in vitro* anti-psoriatic properties in keratinocytes, immune cells and cell-free enzymatic assays.
- The multifaceted anti-psoriatic action of SIRB-001 is executed by targeting all the hallmark features of psoriasis; hyper-proliferation, apoptosis, inflammation and angiogenesis in keratinocyte arm, IL-17/IL-23 inhibition in immune arm and key signaling markers.
- These findings correlate with the anti-psoriatic effect of SIRB-001 in psoriasis patients and provides the scientific proof-of concept for its anti-psoriatic claim.
- Based on the diverse array of *in vitro* anti-psoriatic properties, SIRB-001 presents a promising and clinically useful polyherbal therapeutic agent for psoriasis.
- Further, formulations of SIRB-001 have shown promising *in vivo* anti-psoriatic activity in TPA and IMQ induced animal models.
- 2 clinical studies with SIRB-001 based formulations have been successfully completed, demonstrating excellent anti-psoriatic efficacy.
- Owing to its strong anti-inflammatory potential, SIRB-001 is also being clinically tested for efficacy in other dermatological skin indications, such as eczema and is showing encouraging results.

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