Anti-psoriatic potential and safety of a novel polyherbal formulation

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Disclosure of interests

- There are no conflict of interest declared by the co-authors of this eposter.
- There are no companies, etc., in a relation of conflict of interest requiring disclosure in relation to the eposter.

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Introduction

- Psoriasis is a non-infectious, chronic inflammatory and autoimmune skin disorder, characterized by well-defined erythematous plaques with red, itchy, flaky, crusty and silvery patches.
- Psoriasis is associated with uncontrolled hyper-proliferation of keratinocytes in the epidermis, disturbed apoptosis, over-secretion of cytokines and angiogenic factors.
- There is an unmet need for the development of novel herbal therapies for psoriasis on account of high cost and side effects associated with conventional treatments.
- We have developed a novel aqueous mixture (SIRB-001) of 3 Traditional Chinese Medicine (TCM) based herbs *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.
- SIRB-001 has demonstrated multifaceted antipsoriatic activity *in vitro* by targeting 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.
- The present study was conducted to investigate the *in vivo* anti-psoriatic potential of SIRB-001 (aqueous extract and its cream-based formulation).
- Topical and ocular safety studies were conducted using *in vitro* systems.

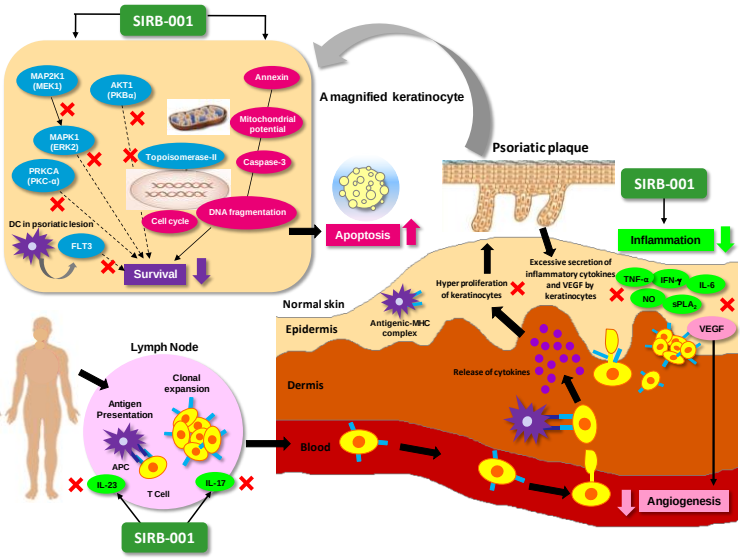

Figure 1 - Putative anti-psoriatic mechanism of action of SIRB-001

Materials and Methods

- **Preparation of SIRB-001 extract**

  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.

- **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.

- **SIRB-001 based Cream**

  SIRB-001 cream was prepared using oil phase (Till oil, Cetostearyl alcohol, Arlacel, Liquid Paraffin, Cresmer Wax EW, Stearyl Stearate, Butyl Hydroxyl Toluene, Propylene Glycol), Preservatives (Sodium Methyl Paraben, Sodium Propyl Paraben), humectants (Glycerol), fragrance (Sandal wood oil), water and active ingredients(herbs).

- **TPA induced inflammation model**

  SIRB-001 was assessed in TPA (Phorbol 12-myristate 13-acetate) induced inflammation model in C57BL/6 male mice (7-9 Weeks, n=7/group) by oral (aqueous extract; 500 mg/kg) and topical (aqueous extract; 1:4 and cream 2.5% and 5%) routes and the combinations administered for 10 days. 20 µL of TPA solution containing 2µg of TPA was applied topically on each ear (10 µL each on ventral and dorsal side) on day 0, 2, 4, 7 and 9. The ear thickness was measured daily using digital caliper. On day 10, ear punch biopsy from right ear (4mm) was collected for histopathological analysis. Remaining ears were stored at -80°C for quantification of inflammatory cytokines (TNF-α, IL-6) in the homogenates using ELISA.

- **IMQ induced inflammation model**

  Anti-psoriatic potential of SIRB-001 was assessed in vivo in IMQ (Imquimod) induced psoriasis model in female BALB/c mice (4-6 weeks, n=5/group). 63 mg of IMQ cream (5%) (containing 3.125mg of IMQ) was applied topically each on dorsal area (shaved back) and right ear of mice for 8 days. SIRB-001 was administered orally at dose of 500 mg/kg, 1000mg/kg , 1500mg/kg and topically (2.5% and 5% cream) for 8 days. Ear thickness was measured daily using digital caliper. On day 9, 4mm ear punch biopsy was collected for histopathological analysis (H & E staining). Remaining ear was excised and stored at -80°C for analysis of inflammatory cytokines (IL-23/IL-17) analysis.

- ***In vitro* skin safety**

- Skin irritation testing of SIRB-001 cream was performed in the EpiDerm™ *in vitro* Skin Model. EpiDerm™ tissues (EPI-200) dosed topically with 30µl of SIRB-001 cream in 6 well plate and rinsed after 60 min of exposure. Cell viability was assessed after 42 h of incubation by MTT assay.

- Ocular irritation was tested using HET-CAM (Hen's Egg Test – Chorioallantoic Membrane) assay (INVITTOX n°96). Fertilised chicken eggs (White leghorn chicken) were incubated until embryonic day 10. After removing the egg shell covering the air cell and cutting through the inner egg membrane, SIRB-001 cream was applied to the CAM for 3 min followed with rinsing. After 30 sec of rinsing, the CAMs were monitored closely for any appearance of haemorrhage and lysis of blood vessels.

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Antipsoriatic activity of SIRB-001 in TPA model

- SIRB-001 (oral/topical) demonstrated significant inhibition (p<0.001) of ear thickness as compared to TPA control. Synergistic effects were observed with oral + topical combination of aqueous extract and cream (5%).

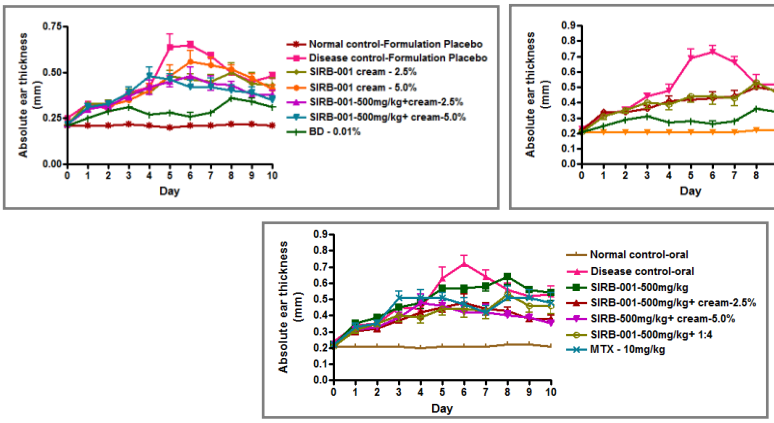


Figure 2 – Inhibition of Absolute ear thickness by SIRB-001 in TPA model

- Oral + topical combination of SIRB-001 aqueous extract and cream (5%) exhibited significant (p<0.05, 0.01) reduction in cytokines (TNF-α, IL-6) in ear homogenates with synergistic effects.

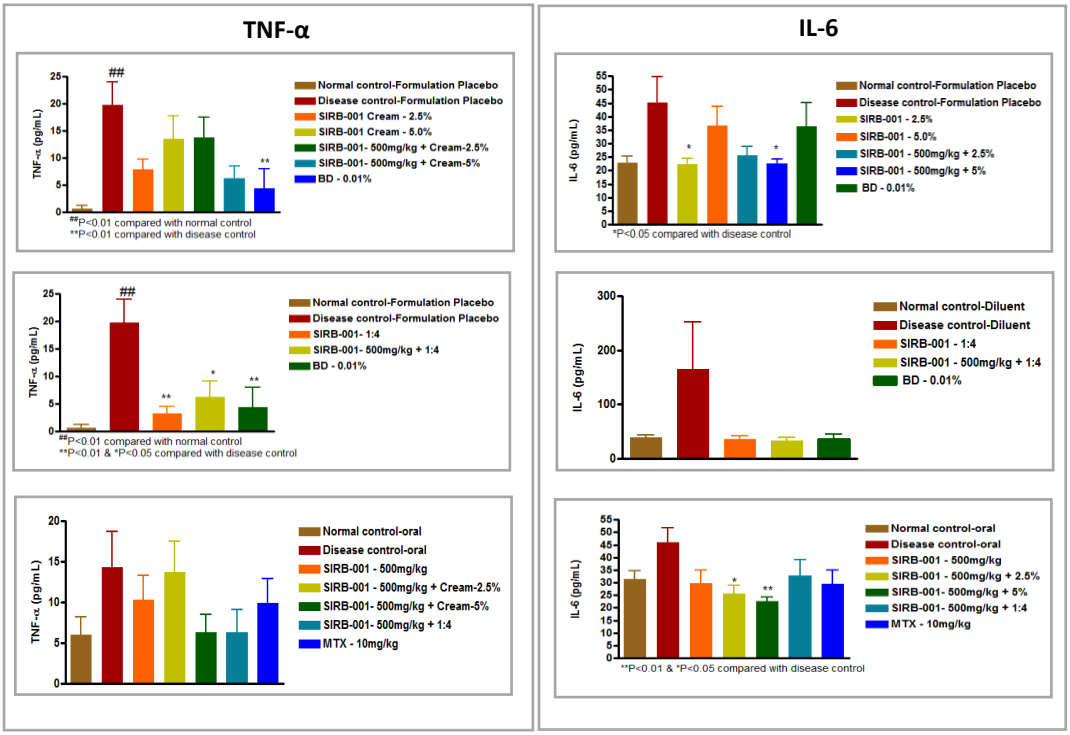
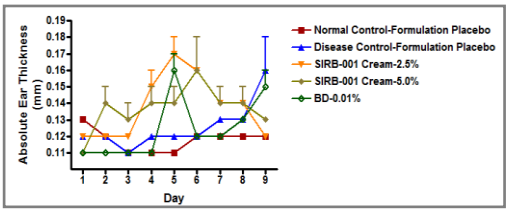


Figure 3 – Downregulation of cytokines in ear homogenates by SIRB-001 in TPA model

Antipsoriatic activity of SIRB-001 in IMQ model

- SIRB-001 aqueous extract (500 mg/kg) and cream (2.5% and 5%) led to significant reduction (p<0.05) in ear thickness (Day-9) and IL-23 levels in ear homogenates.



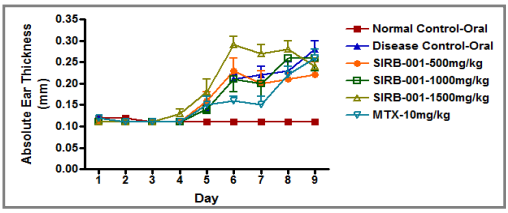
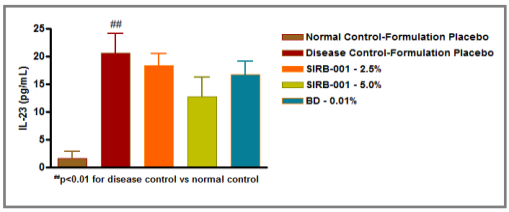


Figure 4– Effect of SIRB-001 on ear thickness in IMQ model



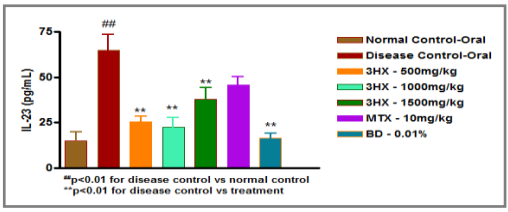


Figure 5– Effect of SIRB-001 on IL-23 secretion in ear homogenates in IMQ model

- SIRB-001, administered orally (500 mg/kg) or topically (2.5%) exerted maximal effects on histopathological end points; hyperkeratosis and inflammatory cell infiltration.

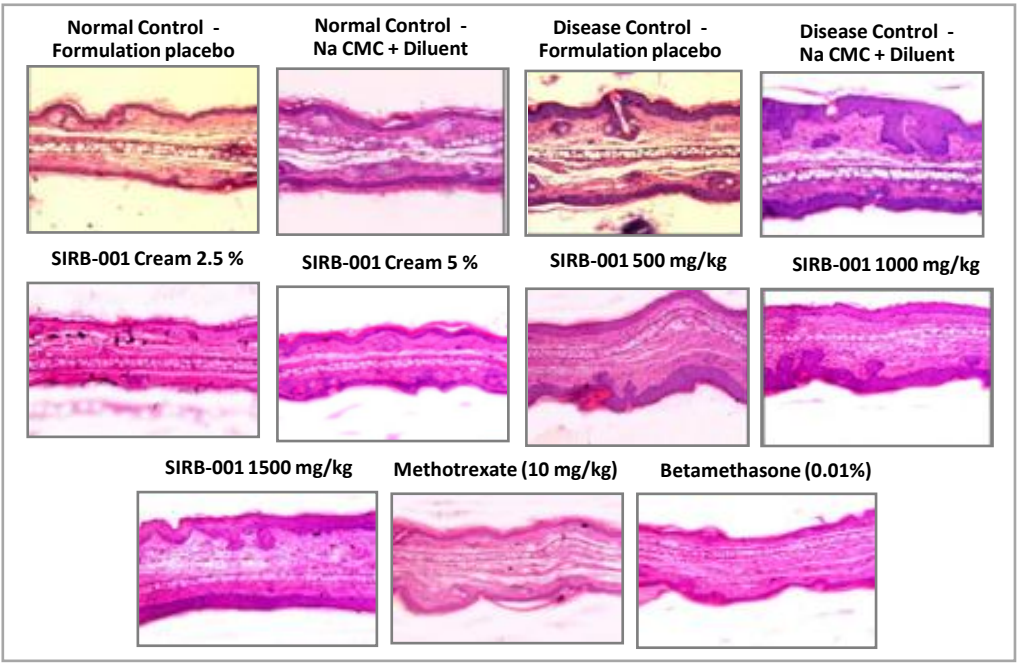


Figure 6 – Effect of SIRB-001 on histopathogical findings (H & E staining)

In vitro safety of SIRB-001 cream

- SIRB-001 cream was tested in EpiDerm™ *in vitro* Skin Model and resulted in >85% of cell viability as compared to control. Hence, SIRB-001 cream was classified as non-irritant.

- SIRB-001 cream was assessed in HET-CAM assay and didn't demonstrate any visible hemorrhage/lysis. Hence SIRB-001 was found to be non irritant based on its CAM appearance.

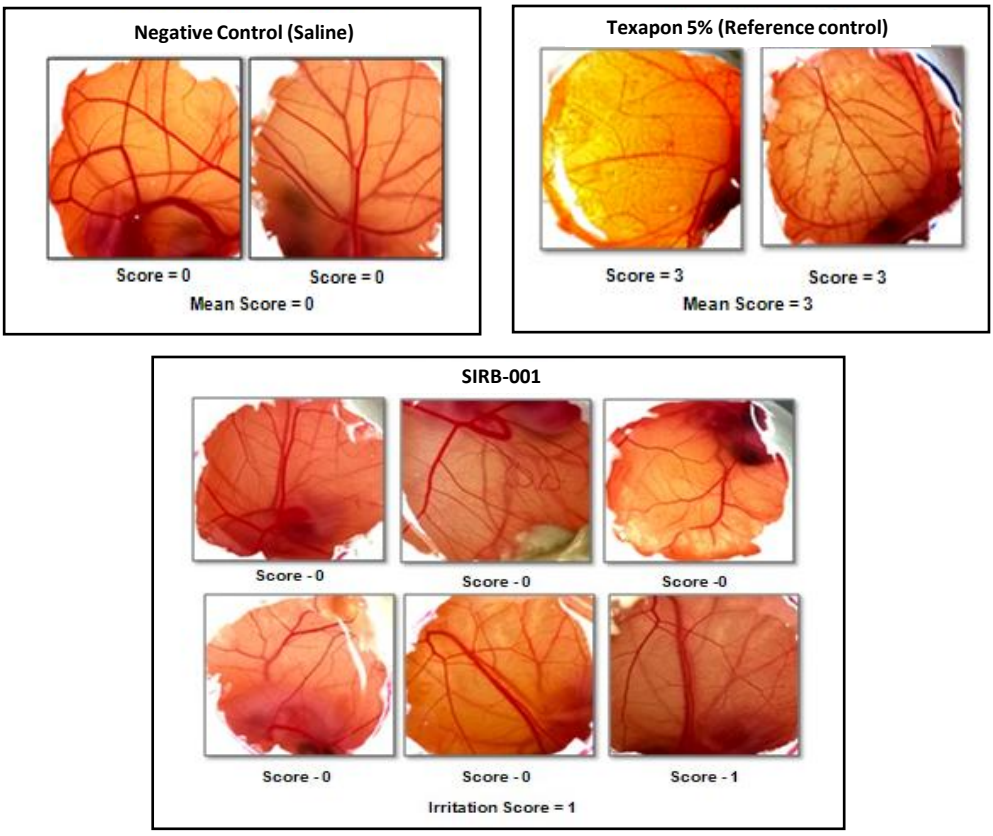
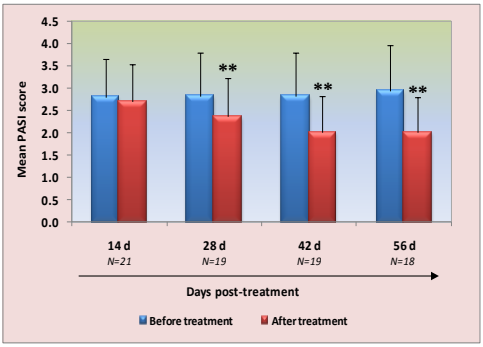


Figure 7- Photomicrographs of CAMs after 3 min of treatment with SIRB-001 cream

Clinical efficacy of SIRB-001 in psoriasis

- The efficacy, tolerability and safety of SIRB-001 Cream was assessed in subjects with psoriatic lesions treated for 4 weeks with twice daily.
- SIRB-001 resulted in significant inhibition of PASI scores
- No safety issue was observed during conduct of the study.



Basic information on Trial

Disease/Target Area	Psoriasis
Product/Route	Polyherbal, Topical
Type of Trial	Observational
Subjects	21
Study Duration	8 Weeks
Primary Endpoint	Tolerability
Region	Germany

Population Characteristics

Age	18-65 Years
Male/Female Ratio	Adequate
Population	German
Severity	Mild to Moderate

Figure 8- Effect of SIRB-001 on PASI scores in psoriasis patients

Clinical efficacy of SIRB-001 in Scalp psoriasis

Basic information on Trial

Product/Route	Polyherbal, Topical
Type of Trial	Observational
Subjects	30 (25 Completers)
Study Duration	8 Weeks
Primary Endpoint	Tolerance & Safety
Secondary Endpoint	Efficacy & Product Acceptability
Region	India

Population Characteristics

Age	**18-65 Years**
Male/Female Ratio	Adequate
Region	Asian, India
Scalp involvement area	10% or greater
Severity (as per IGSS)	At least moderate

- **70% of subjects shows improvement of more than 50% in PSSI.**
- **> 56% of subjects shows improvement of more than 51% or more in terms of VSCAPSI with no serious side effects.**

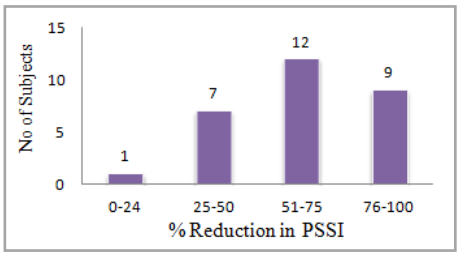


Figure 9 - Effect of SIRB-001 on PSSI scores in psoriasis patients

Conclusions

- SIRB-001 has exhibited remarkable *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 with good safety profile.
- 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 has also been clinically tested for efficacy in other dermatological skin indications, such as eczema and shown promising results.

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