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Evaluation of Anti-Psoriatic Activity of Siddha Formulation *Parangipattai Rasayanam* in HaCaT Cell Line using MTT Assay

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Abstract: *Parangipattai Rasayanam* (PRM) is a well-known Siddha polyherbal formulation. PRM is used for healing purposes traditionally in the management of psoriasis and various other diseases. This prompted us to evaluate the Anti-psoriatic activity of PRM. The aim of this study was to evaluate the Anti-psoriatic activity of aqueous extract of the formulation PRM in HaCaT cell line by using MTT assay. The Antipsoriatic activity of the aqueous extract of PRM was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, using HaCaT cells. For anti-proliferative studies, serial dilutions of PRM (10 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml) were prepared. From the study, it was concluded that the aqueous extract of PRM have significant anti-psoriatic activity.

Keywords: Siddha Medicine, Anti-psoriatic, HaCaT cell line, *Parangipattai Rasayanam*

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Introduction

The Siddha system of Medicine is a traditional medical system, which provides 'Holistic Health'. The Siddha system provides 'preventive, promotive, curative, rejuvenate and rehabilitative' health care with scientific and holistic approach. The word 'Siddha' is derived from the

root word 'Citti', which means attaining perfection, eternal bliss and accomplishment (Thirunarayanan, 2012). Siddha system of medicine is the earliest system of medicine established mostly in Southern parts, especially Tamil Nadu, Puducherry, Kerala and also in Sri

Lanka. The traditional system of medicine has played a vital role in health care in many developing countries.

Nowadays prevalence of dermatological disorders is increasing greatly due to stressful life. One such skin disease is Psoriasis. Nowadays, world's focus turns to herbal medicine because of the side effect of other drugs. Siddha medical system is treating diseases by using herbs, minerals and animal products. Siddha medicine has better remedies for the management of psoriasis. Skin is the largest organ of the body and is easily targeted for allergic and immunological reactions. Skin ailments, specifically psoriasis, dermatitis, urticaria, angioedema etc. are immune-mediated disorders (Meeuwis *et al.*, 2011).

Psoriasis is a common, chronic, autoimmune disease that causes dry, red, scaly patches and flakes to appear on the skin. The prevalence of psoriasis in countries ranges between 0.09% and 11.4%, making psoriasis a serious global problem (Gibbs, 1996). Psoriasis involves the skin and nails and is also associated with a number of comorbidities. The etiology of psoriasis still remains unclear (Danielsen *et al.*, 2013).

The pathogenesis and clinical symptoms of psoriasis include multiple immunological mechanisms and pro-inflammatory cytokines. Herbal drugs can be used as promising antipsoriatic agents. The anti-psoriatic effects of herbal drugs are through modulation of the signaling pathways of the cells. The phytoconstituents inhibit keratinocytes proliferation and differentiation by targeting different molecular targets. The results recommended the use of plants as a safe, effective for treating psoriasis (Bowcock and Cookson, 2004). However, scientific studies to comprehend their anti-psoriatic activity are lacking in the literature.

The aim of this study was to investigate the beneficial role of PRM in the treatment of psoriasis.

Materials and Methods

Standard Operating Procedure of Parangipattai Rasayanam:

Collection of raw drugs:

The raw drugs required for the preparation of 'Parangipattai Rasayanam' were procured from the Country Medicine Store, Parrys, Chennai and from Kanyakumari.

Raw drugs Identification and authentication:

The raw drugs were identified and authenticated by Medicinal Botanist of National Institute of Siddha, Tambaram Sanatorium. The ingredients are: *Chithiramoola ver (Plumbago zeylanica, Linn)*: 35 g; *Sangam ver (Azima tetraacantha Linn)*: 35 g; *Peesangam ver (Clerodendrum inerme Linn)*: 35 g; *Nilappanai kizhangu (Curculigo orchioides)*: 35 g; *Nilakkumilam ver (Gmelina asiatica)*: 35 g; *Poovarasam pattai (Thespesia populnea)*: 35 g; *Amukkara kizhangu (Withania somnifera Dunal)*: 35 g; *Nerunjil ver (Tribulus terrestris)*: 35 g; *Kumilam ver (Gmelina arborea)*: 35 g; *Chukku (Zingiber officinale Roscoe)*: 17.5 g; *Thippili (Piper longum Linn)*: 17.5 g; *Milagu (Piper nigrum Linn)*: 17.5 g; *Omam (Carum copticum)*: 17.5 g; *Sirulavanga pattai (Cinnamomum verum)*: 17.5 g; *Sirunaagap poo (Mesua nagassarium)*: 17.5 g; *Kostam (Costus speciosus)*: 17.5 g; *Citarathai (Alpinia galangal)*: 17.5 g; *Lavanga illai (Syzygium aromaticum)*: 17.5 g; *Sengaththari pattai (Capparis sepiaria)*: 17.5 g; *Inji (Zingiber officinale)*: 17.5 g; *Parangi chakkai (Smilax china Linn.)*: 175 g; Sugar: 350 g; Ghee: 700 g; Honey: 700 g.

Purification processes of ingredients of PRM:

The raw drugs were purified as per the methods mentioned in the Siddha literature. The raw drugs were purified in the *Gunapadam* Lab of the National Institute of Siddha.

Preparation:

All the above-mentioned raw drugs were crushed and made into a fine powder. This powder was then mixed with sugar, honey, and ghee to attain the consistency of *Rasayanam*.

Table: 1 Effect of PRM on Cell viability of HaCaT cell line

S. No.	Concentration (µg/ml)	% Cell Viability
1	10	95.86 ± 0.769
2	50	87.9 ± 4.56
3	100	73.32 ± 1.56
4	150	46.8 ± 4.55
5	200	22.22 ± 1.72

In vitro anti-proliferative activity on HacaT cell lines (Chandane et al., 2021):

The *in vitro* determinations of anti-proliferative effects of the PRM have been performed by counting viable cells after staining with a vital dye. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, which is a water-soluble tetrazolium salt. Upon incubation MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. The resulting-colored solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

Preparation of test solutions:

For anti-proliferative studies, serial dilutions of PRM (10 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml) were prepared. The aqueous extract of the formulation PRM has been utilized for the present study.

HaCaT cell culture and media (Tse et al., 2006):

HaCaT cell lines were procured from NCCS, stock cells were cultured in DMEM medium supplemented 0.07 mM Ca²⁺, 10% heat-inactivated fetal bovine serum, glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 mg/ml) in a humidified atmosphere of 5%

CO₂ at 37°C until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells was checked, centrifuged and was seeded in a 96 well plate and incubated for 24 h – 7 days at 37°C, 5% CO₂ incubator.

Anti-proliferation assay:

For the anti-proliferation assay, 1.25 x 10⁴ HaCaT cells, were seeded per well in 96 well culture plates and incubated overnight. The growth medium was then substituted with fresh medium supplemented with tested compounds at appropriate concentrations. Following incubation for 24 and 48 h till 7 days (to test cell proliferation), the medium was substituted with MTT solution and after a 2 h incubation at 37°C the formazan product was dissolved and absorbance was read at 570 nm using a microplate reader. The optical density of formazan formed in the control and test drug treated wells was taken as a measure of cell viability. IC₅₀ was calculated from dose-response curves.

$$\text{Survival rate (\%)} = \frac{A_{\text{sample}} - A_{\text{b}}}{A_{\text{c}} - A_{\text{b}}} \times 100$$

Results and Discussion

Anti-proliferation assay:

As the concentration of PRM increases, there is a noticeable decrease in cell viability (Table 1, Fig. 1). At lower concentrations (10 µg/ml), the cell viability is high (95.86%), but as the concentration increases, the viability decreases

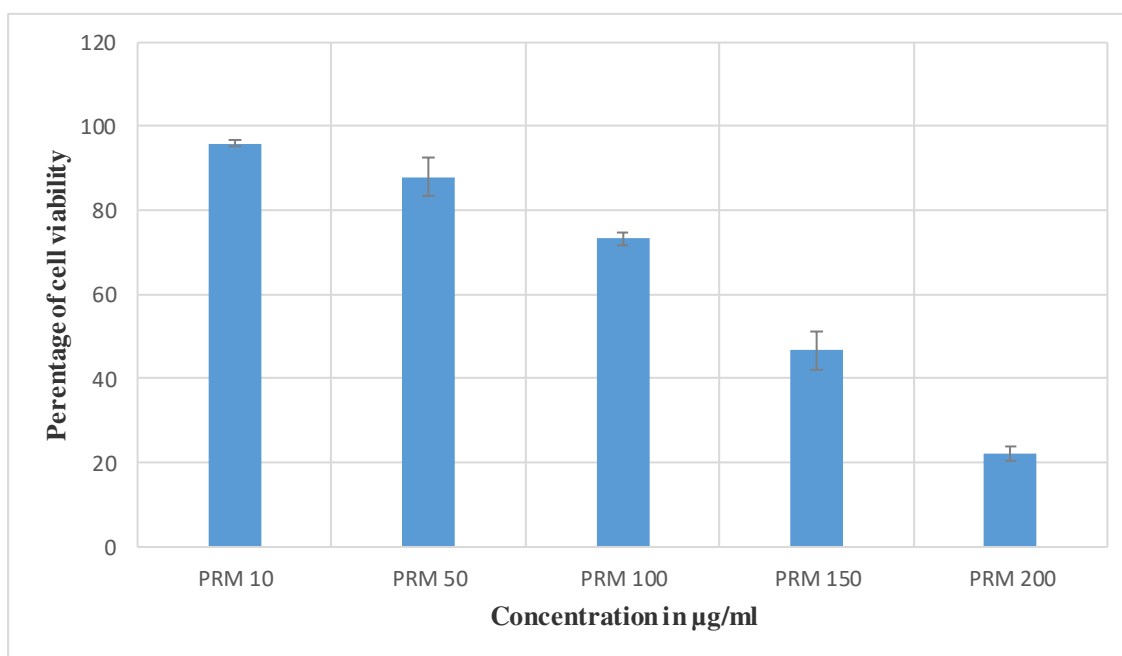


Fig. 1: Effect of PRM on Cell viability of HaCaT cell line.

Table 2: Effect of PRM on Cell death of HaCaT cell line

S. No.	Concentration (µg/ml)	% Cell Death
1	10	4.138 ± 0.76
2	50	12.1 ± 4.56
3	100	26.68 ± 1.56
4	150	53.2 ± 4.55
5	200	77.78 ± 1.72

significantly, with only 22.22% viability observed at the highest concentration (200 µg/ml).

This data suggests that PRM might have a dose-dependent cytotoxic effect on HaCaT cells, meaning higher concentrations of PRM result in more pronounced toxicity to these cells.

The effect of PRM on the cell viability of the HaCaT cell line appears to be dose-dependent, meaning that as the concentration of PRM increases, the viability of the cells decreases. This indicates that PRM may have cytotoxic effects on HaCaT cells, with higher concentrations leading to a more significant decrease in cell viability.

As the concentration of PRM increases, there is

a notable increase in cell death. At lower concentrations (10 µg/ml), the percentage of cell death is relatively low (4.138%), but as the concentration of PRM rises, the percentage of cell death increases significantly, reaching 77.78% at the highest concentration (200 µg/ml).

This data suggests that PRM induces cell death in a dose-dependent manner in the HaCaT cell line, with higher concentrations leading to higher levels of cell death.

The data provided shows effect of PRM on cell death in the HaCaT cell line (Table 2; Fig. 2). As the concentration of PRM increases, there is a notable increase in cell death. This suggests that

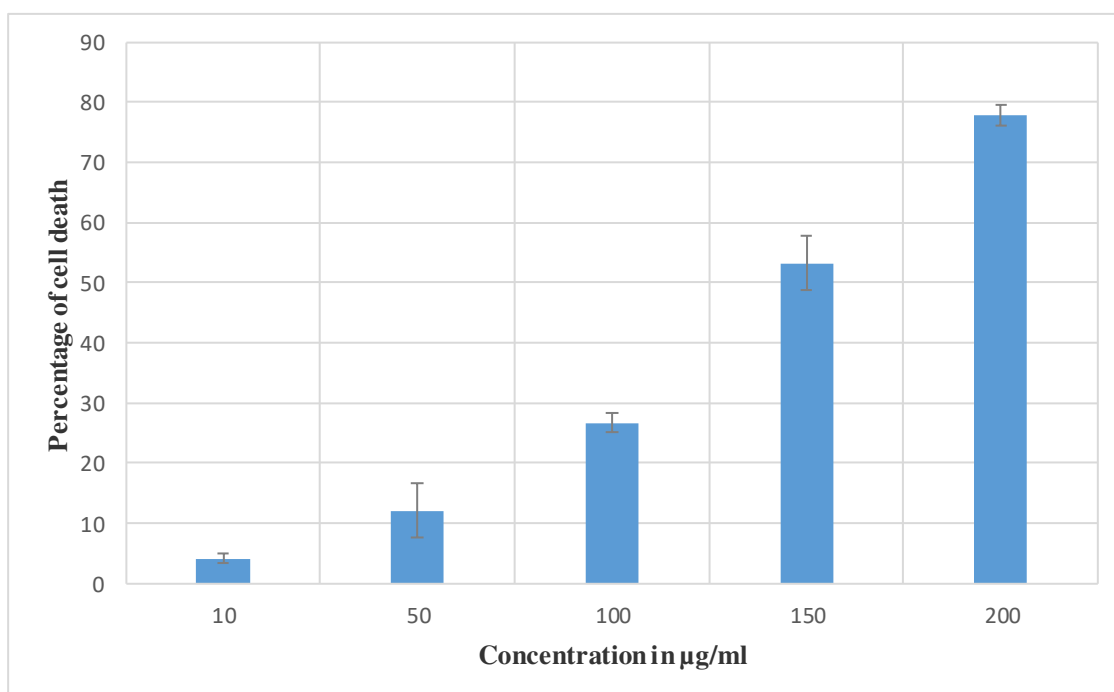


Fig. 2: Effect of PRM on cell death of HaCaT cell line.

Table 3: IC₅₀ Value of PRM

IC₅₀ Value of PRM	129.1 ± 22.57 µg/ml
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PRM may induce cell death in a dose-dependent manner in the HaCaT cell line.

The IC₅₀ value of PRM (half-maximal inhibitory concentration) is observed as 129.1 ± 22.57 µg/ml (Table 3). This means that at a concentration of approximately 129.1 µg/ml of PRM, half of the cells in the HaCaT cell line are inhibited or killed. The range given (± 22.57 µg/ml) indicates the uncertainty or variability associated with this measurement.

In vitro anti-psoriatic evaluation of PRM on the cell viability against HaCaT keratinocyte cell line was performed at different concentrations ranging from 10 µg/ml to 200 µg/ml (Fig. 3). The result obtained from the study revealed that the % of cell viability of HaCaT cell line viability decrease with an increase in the concentration of the PRM. The least viability of cell was observed at the concentration of 200 µg/ml showing

22.22± 1.72%, followed by 150 µg/ml showing 46.8±4.55%, similarly, 100, 50 and 10 µg/ml showing 73.32±1.56, 87.9±4.56 and 95.86±0.769 % cell viability, respectively in MTT assay. The corresponding IC₅₀ value was found to be 129.1±22.57 µg/ml.

Psoriasis has become an important area of research due to its severe effect on the quality of life, cost of treatment, toxicity and side effects of available drugs (Mohan, 2003). Inhibition of excessive proliferation of epidermal keratinocytes is one of the foremost mechanisms by which most of the available anti-psoriatic drugs act. HacaT cells are human spontaneous immortal keratinocyte cells and are often used as an effective model instead of primary-cultured keratinocytes and the data obtained from this model have shown a good correlation with *in vivo* skin irritation (Boukam *et al.*, 1998).

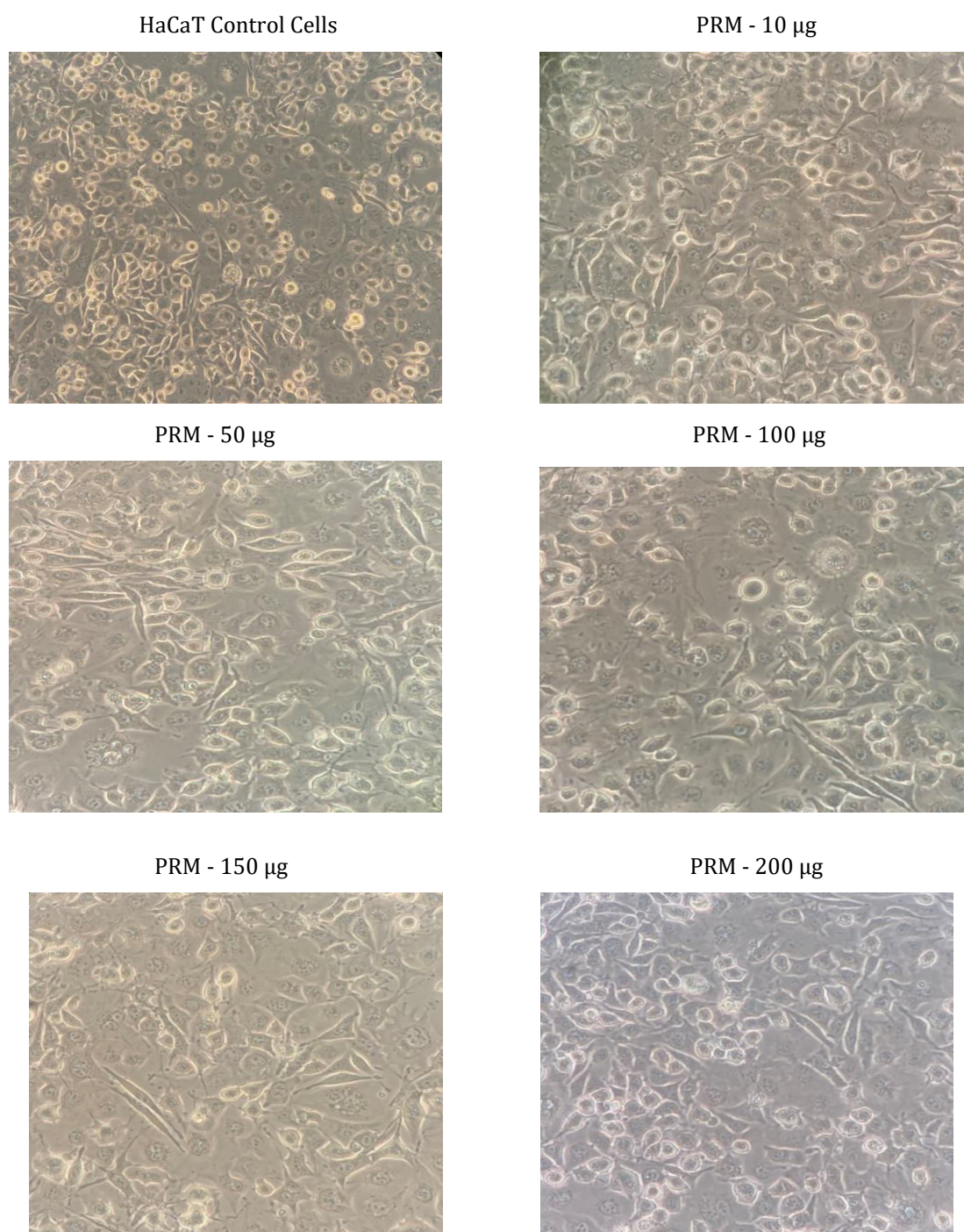


Fig. 3: Cell culture of PRM treated in HaCaT cell lines.

Conclusion

The devastating impact of psoriasis on patients' lives, as well as the high expense of therapy, toxicity, and side effects of current medications, has made psoriasis research a priority. As an alternative to primary-cultured keratinocytes,

HaCaT cells are a human spontaneous immortal keratinocyte cell line that has demonstrated a strong association with results acquired from *in vivo* skin irritation models. This study revealed the significant anti-psoriatic activity of Siddha ploy herbal formulation PRM. These findings

confirmed the claims of the use of PRM in the management of psoriasis.

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