

Anti-Psoriatic Activity of Idi Valladhi Mezhu in Psoriasis (HaCaT) Cell Lines Study - In Vitro

T. Selvi¹, R. Antony Duraichi²

¹PG Scholar, Department of Gunapadam, GSMC, Palayamkottai, Tamil Nadu, India

²Lecturer, Department of Gunapadam, GSMC, Palayamkottai, Tamil Nadu, India

Abstract: Psoriasis is a chronic, multisystem inflammatory disease predominantly involving skin and joints. It has inflammatory and hyperplastic effect on skin, which is characterized by erythema and increased scaly plaques. Psoriasis tends to go through cycles, subsiding or flaring or relapsing, which requires regular and prolonged treatment. It not only affects skin but also social life of the patients causing mental depression and difficult interpersonal relationships. It is common in both male and female genders. Mostly seen in patients with streptococcal infection, psychological stress, etc. It is characterized by infiltration of immune cells, epidermal hyperproliferation, and abnormal keratinocyte differentiation. Conventional (allopathic) drugs are used to treat various auto-immune disorders. The alternative system of medicine such as Siddha medicine is effective in treating of auto-immune disorders. In order to annotate the anti-psoriatic activity of "Idi Valladhi Mezhu" This study aims to demonstrate the anti-psoriatic activity of IVM using Psoriasis (HaCaT) cell lines. This study uses Siddha formulated Idi Valladhi Mezhu as an anti-psoriatic medication in in-vitro HaCaT cell line study. In-vitro anti-psoriatic evaluation of test drug IVM on the cell viability against HaCaT keratinocyte cell line was performed at varying concentration ranges from 10 to 200 µg/ml. The result obtained from the study reveals that the percentage of cell viability of HaCaT cell line viability decreases due to increase in concentration of the test drug IVM. The characteristic effect of psoriasis such as scaly plaques and recurrence rate has substantially decreased as well.

Keywords: Psoriasis, Idi valladhi Mezhu, HaCaT cell, Anti psoriatic, Cytotoxic activity

1. Introduction

Psoriasis is a chronic, multisystem inflammatory disease with predominantly skin and joint involvement. Beyond the physical dimensions of disease, psoriasis has an extensive emotional and psychosocial effect on patients, affecting social functioning and interpersonal relationships. The cause of the disease is not known, but it is believed to be an auto-immune disease. Approximately 15% of Psoriasis patients may subsequently develop Psoriatic arthritis, a potentially debilitating joint condition.

Psoriasis is characterized by infiltration of immune cells, epidermal hyperproliferation, and abnormal keratinocyte differentiation [1]. Many studies have reported various factors contributing to the pathogenesis of Psoriasis, including genetic factors, the immune system, and environmental conditions, thus recognizing it as a multifactorial disease [2, 3].

There are six common Psoriasis types. They are Plaque Psoriasis, Pustular Psoriasis, Guttate Psoriasis, Nail Psoriasis, Erythrodermic Psoriasis, and Inverse Psoriasis. The most common form, plaque Psoriasis, typically has raised red or white scaly skin lesions with a thickened acanthotic epidermis. Key findings in the affected skin of patients with Psoriasis include vascular engorgement due to superficial blood vessel dilation and altered epidermal cell cycle. Such changes are thought to be related to the various inflammatory cytokines released in the inflammatory process, such as tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and interleukin-17 (IL-17). Altered differentiation of psoriatic keratinocytes is characterized by an upregulation of the early differentiation markers (involucrin, small proline-rich proteins, keratin 6, keratin 16, and keratin 17), and down regulation of the late keratinocyte differentiation markers

(filaggrin, loricrin, and caspase-14).

The Skin disorders are brought under the clinical entity "Kuttam" in Siddha system. In Siddha, Psoriasis is termed as Kalanjagapadai (Synonyms: Sedhil Udhir Padai, Sambal Padai, Venparu Sedhil, Sedhil UdhirNoi) and described as a chronic, non - infectious, recurrent, inflammatory disorder of the skin characterized by reddish, slightly elevated patches covered with silvery white scales.

2. Materials and Methods

Idi ValladhiMezhu is prepared according to the procedure mentioned in siddha book "siddha vaidhyathirattu".

MTT-ASSAY

Anti-proliferative activity

The in vitro determinations of anti-proliferative effects of the test formulation 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 MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, 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 test formulation (10, 50, 100, 150 and 200 µg/ml) were prepared. The aqueous extract of the formulation IVM has been utilized for the present study.

HaCat Cell culture and media

HaCaT cell lines were procured from NCCS, stock cells was 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 an 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 are checked, centrifuged and was seeded in a 96 well plate and incubated for 24hrs – 7 days at 37°C, 5% CO₂ incubator [18].

Anti- proliferation assay

For anti- proliferation assay, 1.25 x 10⁴ HaCaT cells, were seeded per well in 96-well culture plates and incubated overnight. Growth medium was then substituted with fresh medium supplemented with tested compounds at appropriate concentrations. Following incubation for 24 and 48 hours till 7 -days (to test cell proliferation), medium was substituted with MTT solution and after a 2-hour incubation at 37°C the formazan product was dissolved and absorbance was read at 570 nm using 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 [19].

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

3.Results

Table 1: Effect of Test drug IVM on Cell viability of HaCaT cell line

S.No	Concentration in µg/ml	% cell Viability
1	10	88.65 ± 2.674
2	50	80.26 ± 5.157
3	100	70.65 ± 4.355
4	150	53.18 ± 2.579
5	200	39.49 ± 4.672

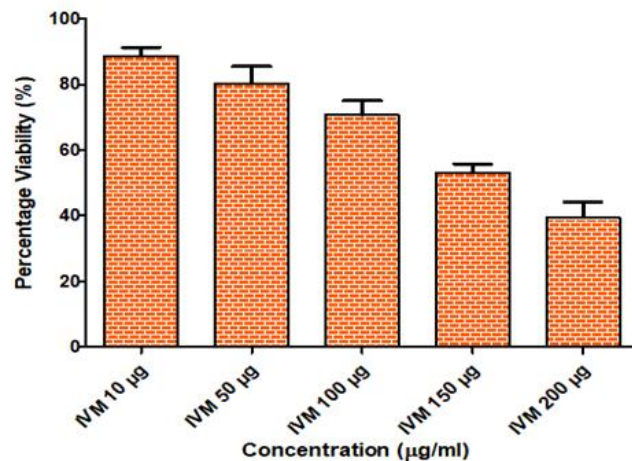


Figure 1: Effect of Test drug IVM on Cell viability of HaCaT cell line

Table 2: Effect of Test drug IVM on Cell death of HaCaT cell line

S.No	Concentration in µg/ml	% cell Death
1	10	11.35 ± 2.674
2	50	19.74 ± 5.157
3	100	29.35 ± 4.355
4	150	46.82 ± 2.579
5	200	60.51 ± 4.672

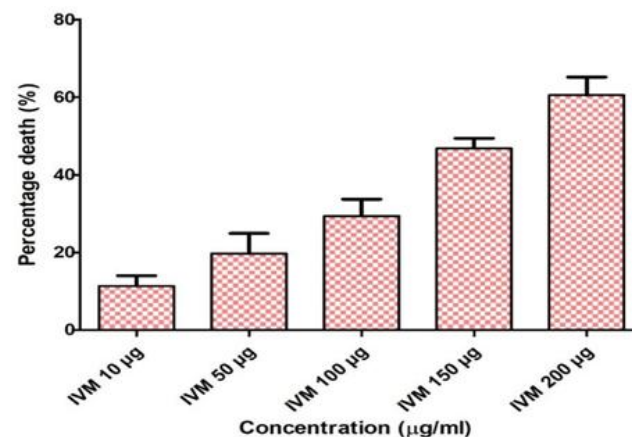


Figure 2: Effect of Test drug IVM on Cell death of HaCaT cell line

IC 50 Value of IVM

IC 50 Value of IVM	165.5 ± 16.25 µg/ml
--------------------	---------------------

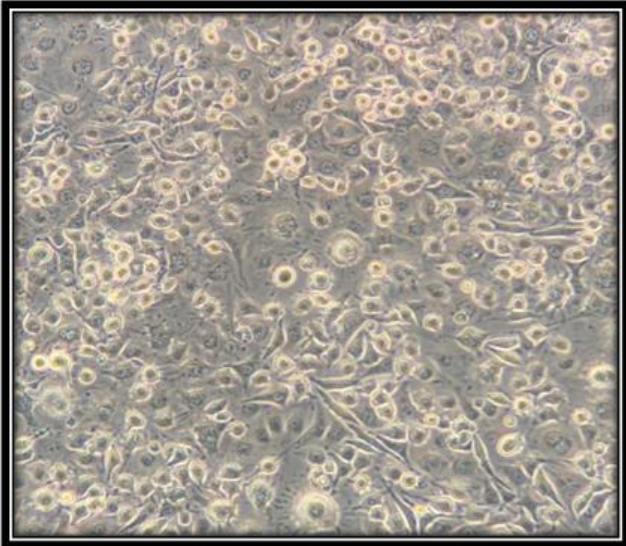


Figure 3: HaCaT Control Cells

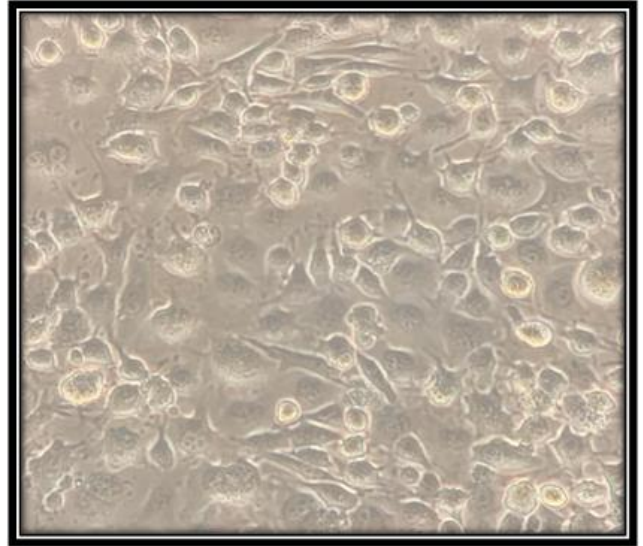


Figure 6: Test Drug IVM - 100 µg

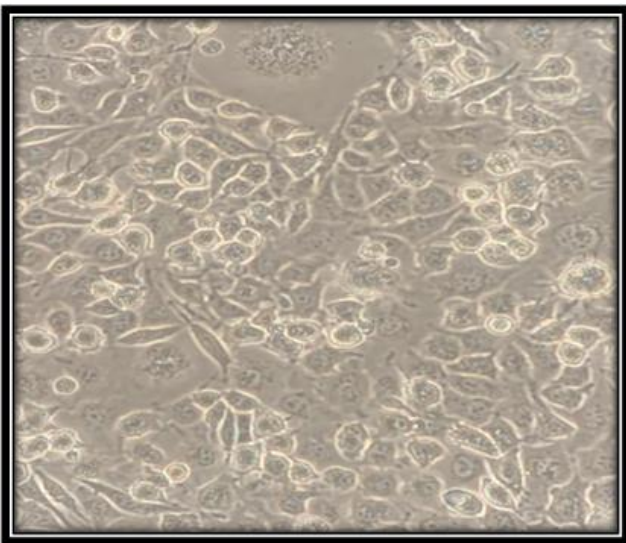


Figure 4: Test Drug IVM - 10 µg

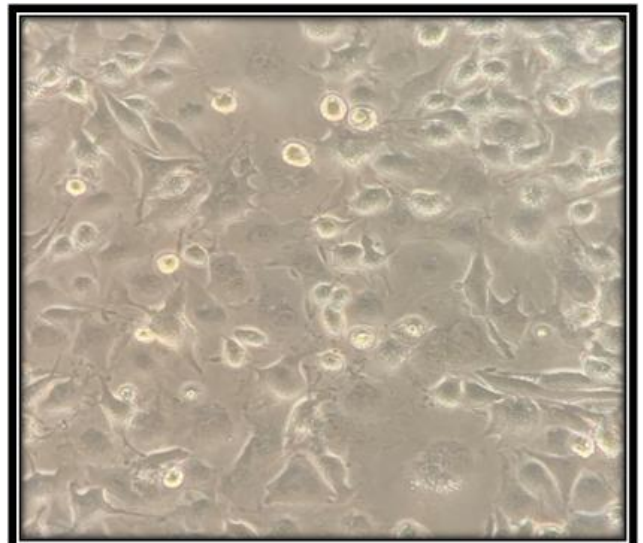


Figure 7: Test Drug IVM - 150µg

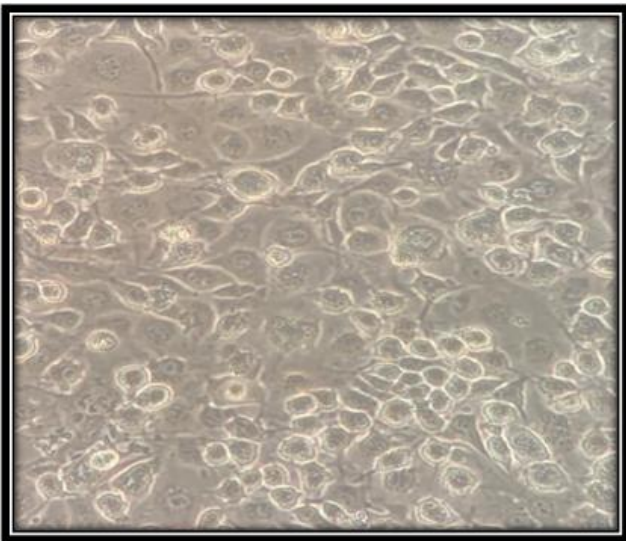


Figure 5: Test Drug IVM - 50 µg

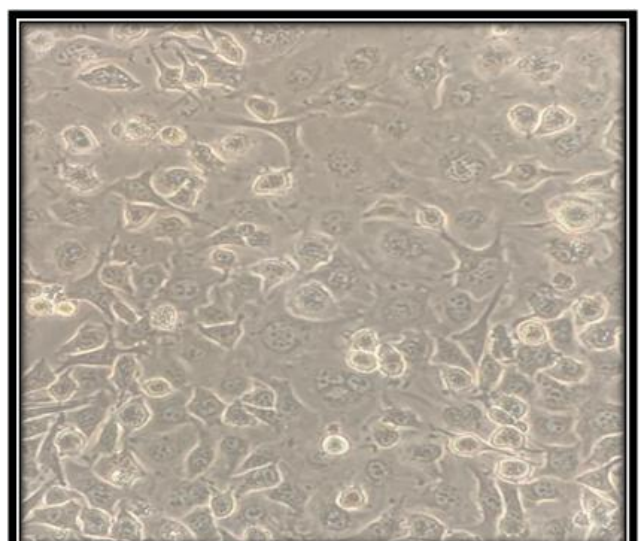


Figure 8: Test Drug IVM - 200µg

4.Discussions

In-vitro anti-psoriatic evaluation of test drug IVM on the

Volume 11 Issue 7, July 2022

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

cell viability against HaCaT keratinocyte cell line was performed at varying concentration ranges from 10 to 200 µg/ml. The result obtained from the study reveals that the percentage of cell viability of HaCaT cell line viability decrease with increase in concentration of the test drug IVM. Least viability of cell was observed at the concentration of 200µg/ml shows 39.49 ± 4.672 %, followed by this 150µg/ml shows 53.18 ± 2.579 %, similarly 100, 50 and 10 µg/ml shows 70.65 ± 4.355 , 80.26 ± 5.157 and 88.65 ± 2.674 % cell viability in MTT assay. The corresponding IC50 value was found to be 165.5 ± 16.25 µg/ml.

5.Conclusion

Treatment of psoriasis majorly involves controlling tissues / skin cells from its accelerated growth and removal of dead cell built-up which causes red, itchy scaly patches / scales.

In Siddha system of medicine, treatment method includes topical therapy as well as oral medication. IVM which is given orally (inter oral medication) has anti proliferative activities. Analyzing the action of IVM is HaCaT cell cultures and media exhibits considerable control of proliferative keratinocyte cells. This can further more prevent the recurrence of disease. Therefore their study can open up new fields in the treatment of psoriasis.

Reference

- [1] Lowes, M.A.; Suarez-Farinas, M.; Krueger, J.G. Immunology of psoriasis. *Annu. Rev. Immunol.* 2014, 32, 227–255. [CrossRef] [PubMed]
- [2] Amigo, M.; Schalkwijk, J.; Olthuis, D.; de Rosa, S.; Paya, M.; Terencio, M.C.; Lamme, E. Identification of avarol derivatives as potential antipsoriatic drugs using an in vitro model for keratinocyte growth and differentiation. *Life Sci.* 2006, 79, 2395–2404. [CrossRef] [PubMed]
- [3] Saelee, C.; Thongrakard, V.; Tencomnao, T. Effects of thai medicinal herb extracts with anti-psoriatic activity on the expression on nf-kappab signaling biomarkers in hacat keratinocytes. *Molecules* 2011, 16, 3908–3932. [CrossRef] [PubMed]
- [4] Dr. R. Thiyagaran, L.I.M., Siddha maruthuvam – Sirappu, Indian medicine & Homeopathy Department, Chennai. 3rd Edition.
- [5] Gonzalez, R.J. and Tarloff, J.B. (2001) Evaluation of hepatic sub cellular Fractions for alamar blue and MTT reeducates activity. *Toxicology in vitro.* 15, 259-9