INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE
ISSN: 2455-944X
www.darshanpublishers.com
DOI:10.22192/ijcrbm Volume 2, Issue 11 - 2017

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2017.02.11.002

In-Vitro Anti-psoriatic and cytotoxicity potential of the Novel Siddha formulation Swarna Pushpa Rasa Chendhuram using human keratinocyte HaCaT cell lines

A. Anitha[*1], M.Mohammad Mustafa[2]

[*1]P.G.Scholar, Department of Sirappu Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

[2] Reader and Head, Department of Sirappu Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

Corresponding Author: **Dr. A.Anitha**, P.G. Scholar, Department of Sirappu Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

E-mail: *aniindira0901@gmail.com*

Abstract

Psoriasis is an autoimmune skin disorder, which affects approximately 2-3% of the population worldwide. The current conventional therapy cannot offer satisfactory clinical results for most of the patients, largely due to the fact that many anti-psoriatic drugs have serious side effects and psoriasis is prone to developing drug resistance after long term exposure. Natural products are used extensively throughout the world, at least in 70% of drugs available in the global market. However, there is a great threat to biodiversity due to overharvesting raw materials for herbal medicines and health care products. Since many medicinal plants are also in the extinct herb list, it is better to adapt mineral-based medicine practiced traditionally in India. An ancient system of traditional Indian medicine '*Siddha'* uses herbs, metals, and minerals. Siddha system of medicine is one of the ancient traditional healing therapies known to the mankind since several centuries. It has numerous novel formulations which benefit the society for various ailments. SwarnaPushpa Rasa Chendhuram (SPRC) is kind of novel herbomineral preparation consist of mercury, sulfur, Stannum, Ammonium chloride and *Benincasahispida*. HaCaT cells are the immortalized human keratinocytes and have been extensively used to study the epidermal homeostasis and its pathophysiology. The main aim of the present investigation is to evaluate the anti-psoriatic and cytotoxic potential of the formulation SPRC using standard optimized HaCaT cell lines. The result obtained from the study reveals that the percentage of HaCaT cell viability decrease with increase in concentration of the siddha formulation SPRC. Least viability of cell was observed at the concentration of 100µg/ml shows 14.92%. Further the highest percentage inhibition of about 85.07% observed at 100µg/ml of SPRC. It was concluded from the present investigation that the siddha formulation SPRC possess significant anti-psoriatic and cytotoxic activity further actual active compounds responsible for potential cytotoxic has to be studied in detail with different models which exactly simulate the cell biology and actual pathophysiology of the psoriasis in humans.

Keywords: Psoriasis, Siddha system, SwarnaPushpa Rasa Chendhuram, HaCaT cell, Anti-psoriatic, cytotoxic activity.

1. Introduction

Psoriasis is a disease resulted from the hyper proliferation and abnormal differentiation of keratinocytes [2]. A successful antipsoriatic drug that targets the epidermis is defined as a compound that ideally shows low toxicity and restores skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation, or both [3]. The granular layer is present in epidermis. It is greatly reduced or almost absent in epidermis of psoriatic leisions [4]. This condition is known as parakeratosis, one of the most important characteristic features of psoriasis [5]. Granular layer formation around the epidermis is known as orthokeratosis condition.

HaCaT cells are the immortalized keratinocyte line [6]. Because HaCaT cells have a high differentiation potential in cell culture based on the expression of various epidermal differentiation markers, this cell line has been widely used as an alternative for evaluating the anti-psoriatic potential of several siddha preparation [7].

Siddha system of medicine pioneering in emphasize the biological activity of the various phytocomponents with respect to the etiology and pathophysiology of various dread full disease emerging in humans and animals. It is evident that there are some medicinal plants used in siddha has potency of acting as an analgesics, anti-microbial, immune modulators, Hepato , neuro and nephron protectant.

Siddha formulations offers tremendous advantage in clinical practice against metabolic and lifestyle disorders including neuro degenerative diseases. Often investigation on siddha preparations attempted on reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically and now several investigation are being made on its preclinical aspect.Patients believed in siddha preparation as it decreases the symptoms by altering the impaired physiology which most of the allopathic medicines fails to do. Further the siddha medicines act mostly by rejuvenation and prevention there by the disease once cured will be treated for ever. By believing this philosophy siddha practitioners judge the pathology of disease by assessing the fundamental elements of the human body. The commonly elemental analysis included evaluation of vadham, pitham and kabam proportions.

Herbs are natural products and their chemical composition varies depending on several factors and therefore varying from people to people, from energetic decoctions to the use of herbal extracts following Western methodologies of mainstream medicine. Traditional medicines has a very long history: it is the sum total of the practices based on the theories, beliefs and experiences of different cultures and times, often inexplicable, used in the maintenance of health, as like in the prevention, diagnosis, improvement and treatment of illnesses. In most of the developing countries traditional medicine become the integral part of the health care hence it gain more popularity in recent decades further the World Health Organization is engaged to establishing the alternate therapy from the traditional origin and implement several strategy of evaluating the efficacy of novel formulation through clinical research and the appraisal of effectiveness of traditional medicine in global aspect [8].

Siddha system has several novel preparation where in the documentary evidence for the same was very limited as measure of creating a monograph and evidence based data's upon the formulation SwarnaPushpa Rasa Chendhuram (SPRC) .The present study aimed to investigate the anti-proliferative properties of SPRC for psoriasis treatment, for their anti-proliferative effects against keratinocytes using cultured HaCaT cells as a psoriasis-relevant experimental mode.

2. Materials and Methods

2.1. Source of raw drugs:

The Required raw materials were procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India .All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai., Tamil Nadu, India

2.2. Ingredients

The siddha formulation *Swarna Pushpa Rasa Chendhuram* comprises of the following ingredients

Purified Rasam (Hydragyrum) -35 g
Purified Gandhagam (Sulphur) - 35 g
Purified Velvangam (Stannum) - 35g
Purified Navacharam (Ammonichloridum) -35 g
Kalyanapoosanikai (*Benincasahispida*) - Quantity sufficient

2.3. Purification

Rasam (Mercury)

35 gms of Mercury was triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the Mercury was boiled with the juice of Kuppaimaeni (1.3 litres) until it is detoxified.

Gandhagam (Sulphur)

Sulphur was placed in an iron spoon. A small quantity of cow's butter was added and the spoon was heated till the butter melts; this mixture was immersed in inclined position in cow's milk. This procedure was repeated for 30 times to get purified Sulphur. Each time, fresh milk was used.

Velvangam (Stannum)

Velvangam was placed in iron spoon and heated. The melted velvangam was then poured into *vitexnegundo* juice and turmeric (*Curcuma longa*). The same procedure was repeated twice.

Navacharam (Ammonium Chloride)

Hot water and filtered was mixed. After self-cooling it was kept in sunlight. Followed by this the salt settles down at the bottom of the vessel.

2.4. Formulation of SwarnaPushpa Rasa Chendhuram [9]

Purified Velvangam was melted and slightly cooled to which purified rasam was added and grounded well. Purified Navacharam and Purified gandhagam are then added and grinded with lemon juice for 12 hrs. Make it into poultices (villai), followed by drying it was kept in small mud pot. Pot was then sealed with 7 layers of mud pasted cloth. New big pot was filled with sand and the small pot was then placed inside the big pot closed with suitable size mud plate. Pots were then ignited for about 12 hoirs. Followed by cooling the product thus formed was again grounded with *Benincasahispida* juice for 3 hrs and dried in moon shade. The end product was powdered, weighed and preserved in an air tight container.

Dose: 130mg, twice daily
Vehicle: Honey
Duration: 48 Days
Indication: Kuttam, Megaranam, Pun puraigal.

2.5. Cell lines and cultural conditions

Human normal skin keratinocyte cell line (HaCaT) received from (National Centre for Biological Sciences, Bangalore, India and were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 in a humidified atmosphere in a CO2 incubator(NBS, EPPENDORF, GERMANY). The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia) for 2 minutes and passaged to T flasks in complete aseptic conditions. The cells were then grown till 60% confluence followed by activation with 1µl LPS (1µg/ml). LPS stimulated HaCaT cells were exposed with different concentrations of test samples such as.6.25,12.5,25,50,100µg/ml from 1mg/ml stock and incubated for 24 hours s. The % difference in viability was determined by standard MTT assay after 24 hours of incubation [10,11].

2.6. Cells seeding

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) wasseeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

2.7. Anti-psoriatic Evaluation:

The cells were then grown till 60% confluency followed by activation with 1µl LPS (1µg/ml),freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µgin 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

2.8. Anti-psoriatic Assay by Direct Microscopic observation:

Entire plate was observed after 24 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

2.9. *In-vitro* cytotoxicity assay

The effect of the siddha formulation Swarna Pushpa Rasa Chendhuram on the viability of HaCaT cell line were determined by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [12,13]. 100µl of cell suspensions in growth medium were plated in 96-well microtitre plate at concentrations of $1x10^4$cells/well and incubated for 48h at 37^oC in a humidified incubator. After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 72 h at 370C. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 20µL of MTT reagent (5mg/ml) was added to each well and the aluminium foil wrapped 96 wells plate was again incubated in CO2 incubator for another 4 hours until purple formazan crystals are visible. The solution in each well including MTT was aspirated and 100µL of buffered DMSO was added to dissolve formazan. The plates were shaken for 5min. Optical density was measured on a microplate ELISA reader at 540nm with DMSO as control. The cytotoxicity was obtained by comparing the absorbance between the samples and control. The percentage inhibition was calculated as follows:

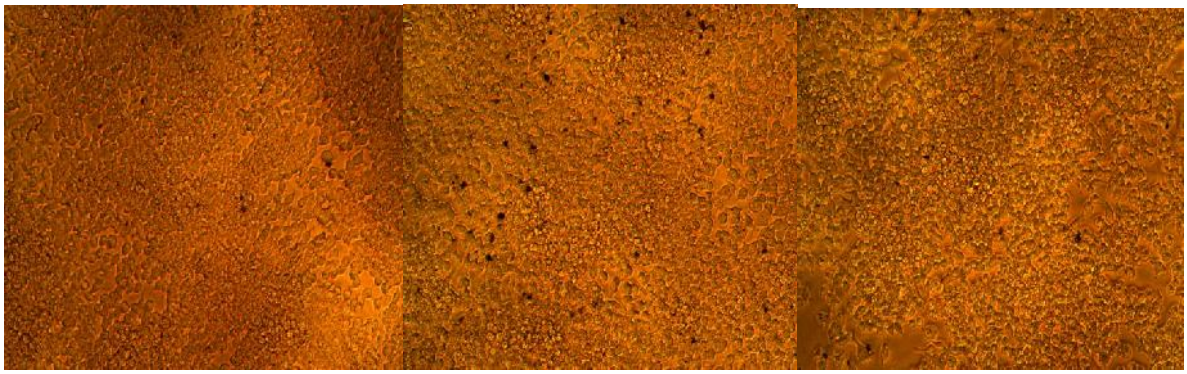
% inhibition = [Abs (control) – Abs (Test)/Abs (control)] x100

3. Results

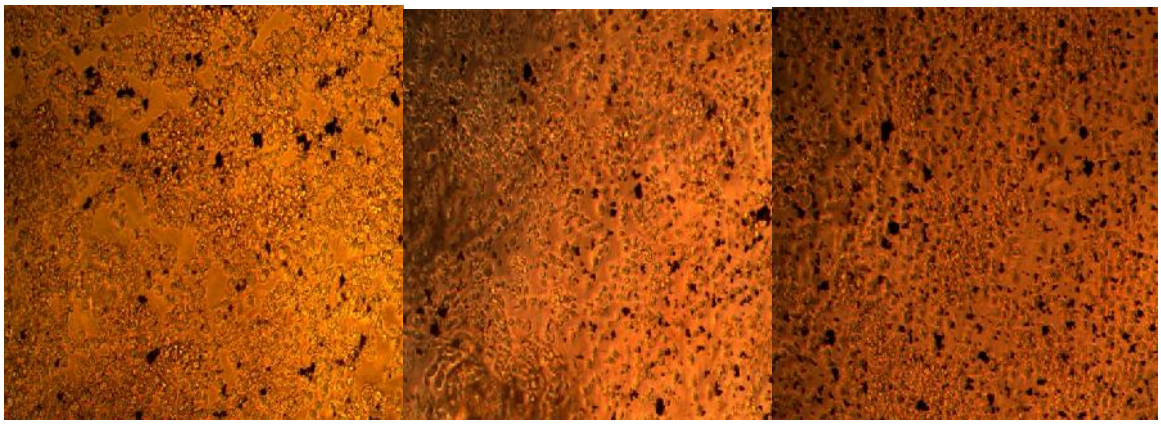
3.2. Proliferation rate of HaCaT Cell line

It was observed from the present investigation that control well treated with LPS at the dose of 1µg/ml shown dense layer of keratinocytes with less intercellular space. Where as well treated with SPRC 6.25 µg/ml shown less viability. SPRC at the dose of 12.5 µg/ml has increased inter cellular space with clumsy appearance as an indication of cell death. There was a significant decrease in cell polulation were observed in wells treated with 25, 50 and 100 µg/ml. In which the highest rate of growth limitation was observed in well treated with 100 µg/ml. As shown in figure 1.

Figure 1: Effect of SPRC in limiting the Proliferation rate of HaCaT Cell line



Control LPS (1µg/ml) **SPRC 6.25 µg/ml** **SPRC 12.5 µg/ml**



SPRC 25 µg/ml **SPRC 50 µg/ml** **SPRC 100 µg/ml**

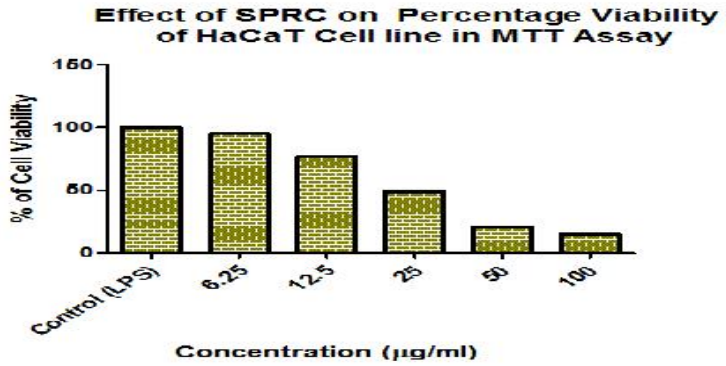
3.2. *In-vitro* cell viability Assay results

In-vitro anti-psoriatic evaluation of the siddha formulation SPRC on the cell viability against HaCaT cell line was performed at varying concentration ranges from 6.25, 12.5, 25,50,100 µg/ml .The result obtained from the study reveals that the percentage of HaCat cell viability decrease with increase in concentration of the siddha formulation SPRC. Least viability of cell was observed at the concentration of 100µg/ml shows 14.92%, followed by this 50µg/ml shows 20.81, similarly 25,12.5 and 6.25 µg/ml shows 48.91, 76.63 and 95.2 % cell viability in MTT assay. As shown in Table 1 and Figure 2.

Table 1: Effect of SPRC on Percentage of Cell Viability of HaCaT Cell line

Control (LPS)	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
100	95.2	76.63	48.91	20.81	14.92

Figure 2: Effect of SPRC on Percentage of Cell Viability

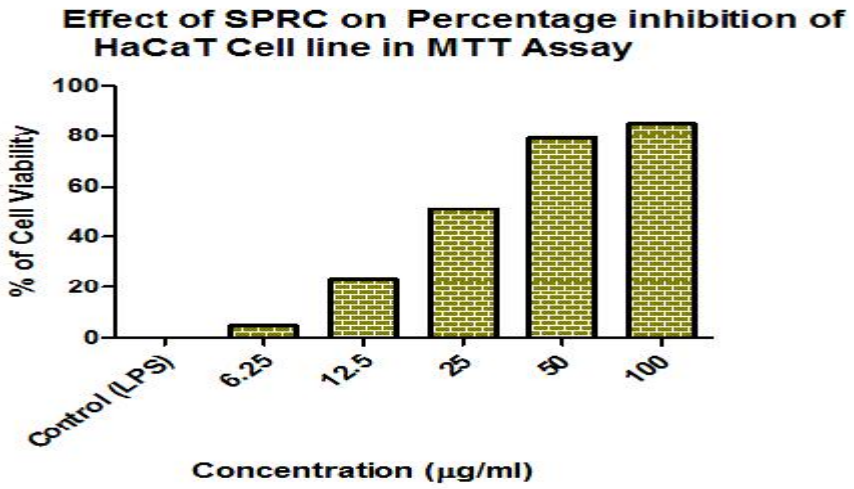


Percentage inhibition of HaCaT cell proliferation increases with increase in concentration of SPRC. Formulationat the concentration of 100µg/ml shows maximum percentage inhibition ofabout 85.07 %. Followed by this 50µg/ml shows 79.18 % similarly 25, 12.5 and 6.25 shows 51.08, 23.36 and 4.78 % inhibition of cell proliferation. As shown in Table 2 and Figure 3.

Table 2: Effect of SPRC on Percentage inhibition of HaCaT Cell line

Control (LPS)	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
0.0	4.78	23.36	51.08	79.18	85.07

Figure 3: Effect of SPRC on Percentage Inhibition



4. Discussion

Psoriasis is a chronic life-long inflammatory disease that primarily affects the skin, musculoskeletal system, the gastrointestinal system and the eye [14].Being an autoimmune disorder no diagnostic tests available for identification of psoriasis [15]. However, the current treatments have not fully met the needs of the sufferers, largely due to the side effects so often associated with various therapies. Also, a large proportion of patients would develop drug resistance after long term drug exposure [16]. Natural remedies seem promising in the management of wide range of dermatological conditions including psoriasis vulgaris [17]. In spite of incredible advances in modern science, technology and allopathic medicine a large we are unable to provide quality healthcare to all. Traditional medicine particularly herbal medicine considered as a major healthcare provider around the globe particularly in rural and remote areas. A large section of people depends on such medicine for their primary healthcare mainly in underdeveloped or developing countries. Indian traditional medicinal system like Siddha, Ayurveda and Unani has a very rich history of their effectiveness; modern research also acknowledged the importance of such medicine. Indian traditional medicine or medicinal plants are also considered as a vital source of new drug [18].

In developing countries, 70-95% of the population relies on herbal medicines for primary care mainly due to cost imperatives or unavailability of conventional drugs. In India, in spite of over 80% of the population dependent upon herbal drugs; it occupies less than 2.5% of the global market share. On the other hand, > 60% market share is being controlled by European Union and North America while 16% being shared by Japan and rest 19% by ASEAN countries [19,20].

Indian system of medicine is one of the oldest and well known documented health traditions in the world. Drug discovery based on traditional information is a key path towards the discovery of new drug. Now a days reverse pharmacology is an approach where discovery of leads/formulations is based on the documented clinical experiences and scientific observations through series of studies. Reverse pharmacology based on traditional knowledge concentrate on the reversing routine ‘laboratory-to-clinic’ development to ‘clinics-to-laboratories’. Safety is considered as most significant point remains and the effectiveness becomes a matter of validation. This process is highly useful to find better and safer leads [21].

Skin comprises essentially three types of cell: keratinocytes, melanocytes and fibroblasts. It is foreseen through wound healing, transplantation and cell culture studies that HaCaT cells may be used as an *in vitro*model for highly proliferative epidermis in tissue engineering. The spontaneously immortalized HaCaT cell line has been a widely employed keratinocyte model due to its ease of propagation and near normal phenotype, but protocols for differentiation and gene delivery into HaCaT cells are extensively found in the literature [22,23,24].

In culture, keratinocyte cells behave in a similar way they do *in vivo*, where cells migrate towards the air interface to form the epithelial surface. Epidermal substitutes require minimum two weeks to expand keratinocytes population. For these reasons, it is necessary to pay heed to the stability of keratinocytes attachment [25]. In the present investigation in-vitro anti-psoriatic evaluation of the siddha formulation SPRC on the cell viability against HaCaT cell line was performed at varying concentration ranges from 6.25, 12.5, 25,50,100 µg/ml .The result obtained from the study reveals that the percentage of HaCat cell viability decrease with increase in concentration of the siddha formulation SPRC. Least viability of cell was observed at the concentration of 100µg/ml shows 14.92%, followed by this 50µg/ml shows 20.81, similarly 25,12.5 and 6.25 µg/ml shows 48.91, 76.63 and 95.2 % cell viability in MTT assay.

HaCaT cells are the immortalized human keratinocytes and have been extensively used to study the epidermal homeostasis and its pathophysiology. T helper cells play a role in various chronic dermatological conditions and they can affect skin barrier homeostasis [26]. Percentage inhibition of HaCaT cell proliferation increases with increase in concentration of SPRC. Formulationat the concentration of 100µg/ml shows maximum percentage inhibition ofabout 85.07 %. Followed by this 50µg/ml shows 79.18 % similarly 25, 12.5 and 6.25 shows 51.08, 23.36 and 4.78 % inhibition of cell proliferation.

5. Conclusion

Traditionally, Siddha formulations like SPRC has been extensively used to treat psoriasis and produced promising clinical results; however, the data’s of the present investigation further justifies the traditional claim of the formulation SPRC. It was concluded from the present investigation that the siddha formulation

SPRC possess significant anti-psoriatic and cytotoxic activity and hence the future research has to be carried out in future to identify the exact mechanism by which the formulation acts in the biological system.

References

1. Min-DukSeo, Tae Jin Kang. HaCaT Keratinocytes and Primary Epidermal Keratinocytes Have Different Transcriptional Profiles of Cornified Envelope-Associated Genes to T Helper Cell Cytokines. BiomolTher (Seoul). 2012; 20: 171–176.
2. Camisa C. Handbook of Psoriasis. Blackwell Science Publisher. USA.1998:114–118.
3. Pol A, Bergers M, Schalkwijk J. Comparison of antiproliferative effects of experimental and established antipsoriatic drugs on human keratinocytes, using a simple 96-well-plate assay. In Vitro Cell DevBiol Anim. 2003;39:36–42.
4. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature. 2007;445:866–73.
5. Danilenko DM. Review paper: Preclinical models of psoriasis. Vet Pathol. 2008;45:563–575.
6. Boukamp P. Petrussevska R. T. Breitkreutz D. Hornung J. Markham A. Fusenig N. E. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell Biol. 1988;106:761–771
7. Grabbe J. Welker P. Rosenbach T. Nürnberg W. Krüger-Krasagakes S. Artuc M. Fiebiger E. Henz B. M. Release of stem cell factor from a human keratinocyte line, HaCaT, is increased in differentiating versus proliferating cells. J. Invest. Dermatol. 1996; 107:219–224.
8. Fabio Firenzuoli. Herbal Medicine Today: Clinical and Research Issues. eCAM 2007;4:37–40
9. Sikicharathinadeepam –kannuswamypillai page no:240
10. Brandner JM. Tight junctions and tight junction proteins in mammalian epidermis. Eur J Pharm Biopharm. 2009;72:289–294.
11. Brandner JM, Kief S, Grund C, Rendl M, Houdek P, Kuhn C. Organization and formation of the tight junction system in human epidermis and cultured keratinocytes. Eur J Cell Biol. 2002;81:253–263.
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65:55–63.
13. Kiruthiga R, Sivaraman D.Evaluation of Anti-Cancer Potential Of Indian Medicinal Herbs MorindaTinctoria And FicusHispida Using Hela Cell Line By MTT Assay Method. World Journal of Pharmacy and Pharmaceutical Sciences.2016; 5: 1658-1670.
14. Raychaudhuri SP, Gross J. A comparative study of pediatric onset psoriasis with adult onset psoriasis. PediatrDermatol. 2000;17:174–8.
15. Ortonne JP. Aetiology and pathogenesis of psoriasis. Br J Dermatol. 1996; 135:1–5.
16. Lebwohl M, Ali S. Treatment of psoriasis. Part 1. Topical therapy and phototherapy. J Am AcadDermatol. 2001;45:487–498.
17. Brown DJ, DattnerAM. Phytotherapeutic approaches to common dermatologic conditions. Arch Dermatol. 1998;134:1401–1404.
18. Saikat Sen. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. J Tradit Complement Med. 2017; 7: 234–244.
19. WHO Traditional medicine strategy 2002–2005. Geneva: World Health Organization, “Global review”. 2002: 66.
20. Guidelines on registration of traditional medicines in the WHO African Region. Brazzaville: World Health Organization Regional Office for Africa,” Background and Purpose. 2004: 40.
21. Patwardhan B, Mashelkar RA. Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? Drug Discov Today. 2009;14:804–811.
22. Lehmann B. HaCaT cell line as a model system for vitamin D3 metabolism in human skin. J. Invest. Dermatol. 1997;108:78–82.
23. Boukamp P, Petrussevska RT, BreitkreutzD.Normal keratinization in a spontaneously immortalized aneuploid keratinocyte cell line. J. Cell Biol. 1998;106:761–771.
24. Garcia JL. Enhanced keratinocyte cell attachment to atelocollagen thin films through air and nitrogen plasma treatment. Prog. Colloid Polym. Sci. 2011;138:89–94.

25. Metcalfe AD, Ferguson MWJ. Tissue engineering of replacement skin: The crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J. R. Soc. Interface. 2007;4:413–437.
26. Min-DukSeo.HaCaT Keratinocytes and Primary Epidermal Keratinocytes Have Different Transcriptional Profiles of Cornified Envelope-Associated Genes to T Helper Cell Cytokines.BiomolTher (Seoul). 2012; 20: 171–176.

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How to cite this article:
A. Anitha, M.Mohammad Mustafa. (2017). *In-Vitro* Anti-psoriatic and cytotoxicity potential of the Novel Siddha formulation Swarna Pushpa Rasa Chendhuram using human keratinocyte HaCaT cell lines. Int. J. Curr. Res. Biol. Med. 2(11): 11-18.
DOI: http://dx.doi.org/10.22192/ijcrbm.2017.02.11.002