Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(18):12-20
(http://www.derpharmachemica.com/archive.html)

Antipsoriatic Activity of Gel Containing Methylsulphonylmethane Powder and Seed Oil of *Pongamia pinnata* Linn

Salunke Pradnya Balasaheb[1*], Kadam Satwashila Shahajirao[1], Patil Sandeep Balvant[2], Kolap Mahesh Babasaheb[3]

[1]Late Narayandas Bhawandas Chhabada Institute of Pharmacy, Raigaon Satara, Maharashtra, India
[2]Dr. Shivajirao Kadam College of Pharmacy, Digraj Sangli, Maharashtra, India
[3]Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

ABSTRACT

To develop gel using Methylsulphonylmethane powder and seed oil of Pongamia pinnata Linn to minimize side effects of current therapy. All prepared formulations were evaluated for pH, viscosity, spreadability, extrudability. The release of optimized batch was carried out using cellophane membrane into phosphate buffer 6.8 at 37°C. The characterization was carried out by using Fourier Transform Infra-Red (FTIR), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscope (SEM) analysis. In vitro and in vivo activities were performed by using HaCaT cell line and Mouse tail model respectively.

Keywords: Psoriasis, Orthokeratosis, *Pongamia pinnata*, Methylsulphonylmethane

INTRODUCTION

Psoriasis is ancient common, chronic, inflammatory, autoimmune dermatitis. Psoriasis is a common disorder in which environmental factors contribute to the development of sharply demarcated erythematous scaly plaques in genetically predisposed. It is characterized by epidermal keratinocytes hyper proliferation, abnormal keratinocytes differentiation, erythematous, sharply demarcated papules and rounded plaques, covered by silvery micaceous psoriasis is one of the most common non-infectious skin disease affecting upto 2.5% of world population. It is uncommon under the age of 10 years, it appears between age 15-30 years affecting men and women. *Pongamia pinnata* Linn and methylsulphonylmethane (Sulphur powder) used in many dermatological disorders like eczema, scabies, psoriasis from ancient time. A gel has good elasticity, satisfactory bioadhesion and is without irritation or sensitizing action. Natural or synthetic polymers that are biodegradable may offer advantage over non-biodegradable polymers for topical drug delivery applications [1].

MATERIALS AND METHODS

Materials

Methylsulphonylmethane and *Pongamia pinnata* oil purchased from Loba Chemie Pvt. Ltd. Mumbai & local area of Sangli respectively. All the materials, solvents and chemicals were of analytical grade.

Preparation of formulations

Accurately weighed quantity of Carbapol 934 was taken in a beaker and dispersed in 80 ml of distilled water. Kept the beaker aside to swell Carbapol 934 for 24 h and then stirring should be done using mechanical stirrer at 1200 rpm for 30 min. Take 5 ml of propylene glycol and required quantity of methylparaben and propylparaben to it and kept in distilled water aside for 20 min and then stirred properly. In small beaker take drug and seed oil of *P. pinnata* and kept on sonicator for sonication. Finally after all carbapol dispersed solution in beaker was mixed and kept for stirring at 800 rpm for 20 min and then drug was added. Eventually triethanolamine was added drop wise to formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency [1,2].

Formulation of gel: The 5 different batches were developed as below (Table 1).

Table 1: Different batches of gel formulation

Composition	B1	B2	B3	B4	B5
Methylsulphonylmethane (mg)	1.3 g	1.3 g	1.3 g	1.3 g	1.3 g
Pongamia pinnata Linn oil (ml)	5 ml	5 ml	5 ml	5 ml	5 ml
Carbapol 934 (g)	0.5	1.0	1.25	1.50	1.75
Propylene Glycol (ml)	5	5	5	5	5
Methylparaben (mg)	0.2	0.2	0.2	0.2	0.2
Propylparaben (mg)	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	100	100	100	100	100
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.	q.s.

Evaluation of prepared gel

Physical appearance and homogeneity: The prepared gel formulations were inspected visually for their colour, homogeneity (Table 2).

pH: To determined pH, 1 g of each gel formulation were transferred into 100 ml beaker with distilled water and pH measured by using digital pH meter (Table 3).

Viscosity: A Brookfield Viscometer RVT with spindle no.64. The spindle was rotated at 10 rotations/min and samples were allowed to settle over 30 min at temperature 25 ± 1°C before measurements were taken according to Misal et al. (Table 4).

Spreadability: It was determined by using spreadability apparatus (Table 5).

Extrudability study: The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 50 g was placed over the slide and then the cap was removed (Table 6).

Optimization of batch: The batches were optimized by checking, and by studying physical evaluation of their pH, viscosity, spreadability and extrudability of all formulations batches. By studying the evaluations parameters of all batches, from gel formulation the optimized batch was decided and was further studied.

Evaluated parameter of optimized batch

In vitro diffusion study

The diffusion studies of the prepared gel can be carrying out in Franz diffusion cell for studying the dissolution release of gel through a synthetic cellophane membrane was used as diffusion membrane. Gel sample (0.5 g) was taken in cellophane membrane and the diffusion studies were carried out at 37 ± 0.5°C using 250 ml of phosphate buffer (pH 7.4) as the dissolution medium. 5 ml of each sample was withdrawn periodically at 1, 2, 3, 4, 5, 6, 7 and 8 h. And each sample was replaced with equal volume of fresh dissolution medium. Then the samples were analyzed for the drug content by using phosphate buffer as blank. The absorbance was taken by UV spectroscopy.

Skin irritation study

The Wistar rats of either sex of weight 150-200 g were used for this test. The intact skin was used. The hairs were removed from the back of rat 3 days before experiment. Formulated gel was applied on test animal. Gel base was applied on the back of animal as control. The animals were treated daily upto seven days and finally treated skin was examined visually for erythema and edema [3].

Stability study

Stability testing of drug product being as a part of drug discovery and ends with the commercial product, to assess the drug and formulation, stability study were done. Optimized gel formulation (50 g) was packed in the wide mouth plastic container and subjected to accelerated stability study at 40 ± 10°C and 75% RH for a period of 3 months as per International Conference on Harmonization (ICH) guidelines. The gels were withdrawn and evaluated for physical changes in colour, odour, consistency, pH, viscosity and spreadability study.

Fourier transform infra-red (FTIR) analysis

It is the powerful tool for the determination of functional group. IR of formulated was done by using FTIR Agilent technology model Cart 630.

Scanning electron microscopy (SEM)

The surface topography, particle size, morphology of the gels were investigated with a SEM. SEM is one of the common methods used owing to the simplicity of sample preparation and ease of operation. The analysis was performed by placing samples on a brass stub using a double-sided adhesive tape and was made electrically conductive by coating in vacuum (6 pas) with platinum using ah Ion Sputter at 15 mA. The microphotographs were taken at an excitation voltage of 10 kv.

Methods

In vitro methods

HaCaT cell line (psoriatic skin cell line): *In vitro* antipsoriatic study was performed using Sulforhodamine B (SRB) assay. HaCaT human keratinocyte cell (Procured from NCCS Pune) lines were used. Culturing of cell lines was done in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). The monolayer cell culture was trypsinized and the cell count adjusted to 1.0×10^5 cells/ml using growth medium in a 96-well microtitre plate, 0.1 ml of the diluted cell suspension (Approximately 10,000 cells/well) was added. After 24 h, the monolayer was washed once, when a partial monolayer was formed, the supernatant was taken, and 100 µl of drug dilution prepared with above media was added per well in microtitre plates. The plates were microscopic examination was carried out and observations were recorded for every 24 h. After 72 h, 25 µl of 50% Trichloroacetic Acid (TCA) was added to the wells such that it forms a thin layer over the drug dilutions to form overall concentration of 10%. The plates were then incubated at 4°C for 1 h.

The plates were flicked; culture was washed five times with tap water to remove traces of medium, drug and serum, and was then air dried. The

air-dried plates were stained with SRB for 30 min. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100 µl of 10 mM tris buffer.

Percent cytotoxicity=Reading of control–Reading of treated cells/Reading of control × 100

In vivo method

Perry scientific mouse tail method for psoriasis: The mouse Tail Test first described by Jarrett and Spearman (1964) with certain modifications reported by authors is a morphometry-based, sensitive and well reproducible method. It allows the quantitative evaluation of the effects of antipsoriatic drugs on epidermal differentiation crucially disturbed in psoriasis. This model is based on the induction of orthokeratosis in those parts of the adult mouse tail which have normally a parakeratosis differentiation.

Albino mice of weight 25-27 g were divided into three groups of six each. Proximal parts tails were treated locally with 1 g of drug, the first group was control which was untreated and second group was standard treated with 0.05% Retino A cream. The Third group was treated with prepared gel. For the topical application gel is applied by the cotton topically to the proximal part of the tail about 2.5 cm and allowed to remain in contact for 2 h a plastic cylinder is slipped over tail and fixed with adhesive tape. Then tails were washed with water. Treatment was given once daily for 14 days. The animals were scarified using deep ether anesthesia by cervical dislocation, 2 h after the last treatment and the proximal parts of their tails were cut and each group tails were stored in treatment and the proximal parts of their tails were cut and each group tails were stored in separate containers containing 10% formalin in buffer solution [3,4].

RESULTS

Physicochemical evaluation of gel formulation

Physical appearance and homogeneity

Table 2: Physical appearance and homogenecity of different batches of gel formulations

Batches	Appearance	Homogeneity
B1	White	Good
B2	White	Good
B3	White	Good
B4	White	Good
B5	White	Good
Standard drug (Retino A 0.05% cream)	White	Good

Measurement of pH

Table 3: pH of different batches of gel formulations of pH

Batches	pH
B1	6.6
B2	6.8
B3	6.5
B4	6.6
B5	6.6
Standard drug (Retino A 0.05% cream)	6.9

Viscosity study

Table 4: Viscosity of different batches of gel formulations

Batches	Viscosity (cps)
B1	4098
B2	4476
B3	4235
B4	5015
B5	5094
Standard drug (Retino A 0.05% cream)	4376

Spreadability study

Table 5: Spreadability of different batches of formulated gel

Batches	Spreadability (g.cm/sec)
B1	19.35
B2	22.50
B3	20.56
B4	16.67
B5	18.36
Standard drug (Retino A 0.05% cream)	23.00

Extrudability study

Table 6: Extrudability of different batches of gel formulations

Batches	Weight of formulations in tube (g)	Weight of gel extruded (g)	Extrudability amount (%)	Appearance
B1	20	17.09	85.45	++
B2	20	18.49	92.45	+++
B3	20	18	90.65	++
B4	20	16.53	82.65	++
B5	20	16.97	84.85	++

Optimization of batch

After analysis of all batches of formulations by their evaluation parameters like appearance and homogeneity, pH, viscosity, spreadability and extrudability, the formulation batch B2 showed good results than other batches. Hence the optimized batch was used for further study like *in vitro* diffusion study. *In vitro* and *in vivo* study, the study of optimized batch was as follows in Tables 7 and 8:

Table 7: Evaluation parameters of optimized batch B2

Optimized batch	pH	Viscosity (cps)	Spreadability (g.cm/sec)	Extrudability (%)
B2	6.8	4476 ± 0.5	25.02 ± 0.001	92.45

Table 8: Drug content of prepared formulations

Formulations	Absorbance (y)	% Drug content
B1	0.5826	91.01
B2	0.5871	91.76
B3	0.5856	92.01
B4	0.5995	93.89
B5	0.6079	95.26

***In vitro* diffusion study**

In vitro diffusion study was performed for optimized batch B2. The results were depicted in table. Batch B2 showed 76.50% drug release through cellophane membrane after 6 h (Table 9).

Table 9: Percentage drug release of optimized batch B2

Time (h)	% Drug release
0	0
1	19.5
2	32.05
3	43.65
4	57.09
5	63.04
6	77.6

Skin irritation study

Absence of skin irritation of gel formulation is acceptable by patient. Skin irritation test performed *in vivo* test on Swiss albino rats. Gel formulation of batch B2 was found to be free from irritation. Observations indicate acceptability of this gel for topical use (Table 10).

Table 10: Skin irritation study of optimized batch B2

Batch	No. of group	Erythema	Edema
B2	Control	0	0
B2	Test	0	0

Stability study

Accelerated stability study of batch B2 indicated that the physical appearance, pH, viscosity, extrudability, spreadability in the prepared gel remained unchanged upon storage for 3 months (Table 11).

Table 11: Stability profile of optimized batch B2 gel formulation

Evaluation	Initial	After1 month	After 2 months	After 3 months
pH	6.7	6.7	6.8	6.8
Viscosity (cps)	3612	3622	3624	3625
Extrudability (g.cm/sec)	92.45	92.37	92.30	92.20
Spreadability	19.50	18.90	18.77	18.69

IR analysis

FTIR analysis of formulated gel (Table 12 and Figure 1)

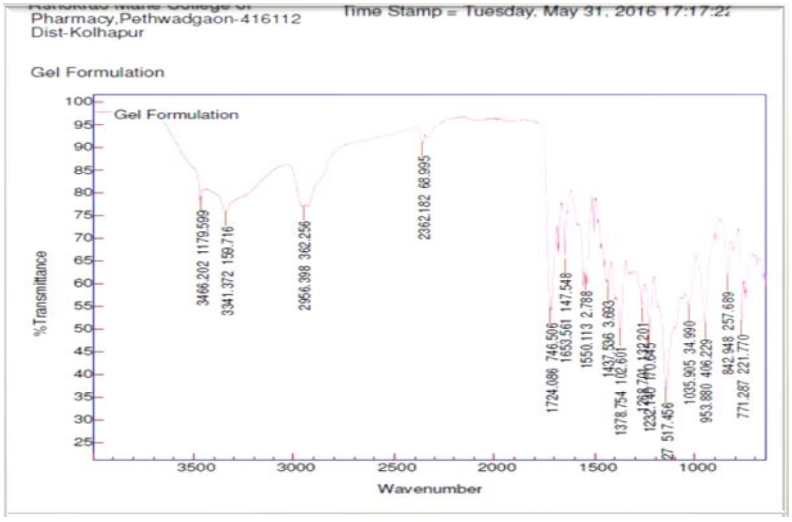

Figure 1: IR spectra of formulated gel

Table 12: IR spectra wavenumber and functional groups

S. No.	Observed peak (cm^{-1})	Peak reported (cm^{-1})	Bond	Functional group
1	1437	1400-1600	C=C stretch	Alkene
2	1724	1670-1820	C=O stretch	Carbonyl
3	2956	3000-3100	-OH stretch	Carboxylic acid
4	1149	1225-980	S=O stretch	Sulphonyl
5	1232	1320-1000	C-O-C	Ethylene oxide

Differential scanning calorimetry (DSC)

The DSC thermo gram shows the endothermic peak at its melting point i.e., 92.48°C (Figure 2). This indicates the possible change in melting point, release kinetics. Surface topography, particle size, morphology of the gels was investigated with a SEM. SEM is one of the common methods used owing to the simplicity of sample.

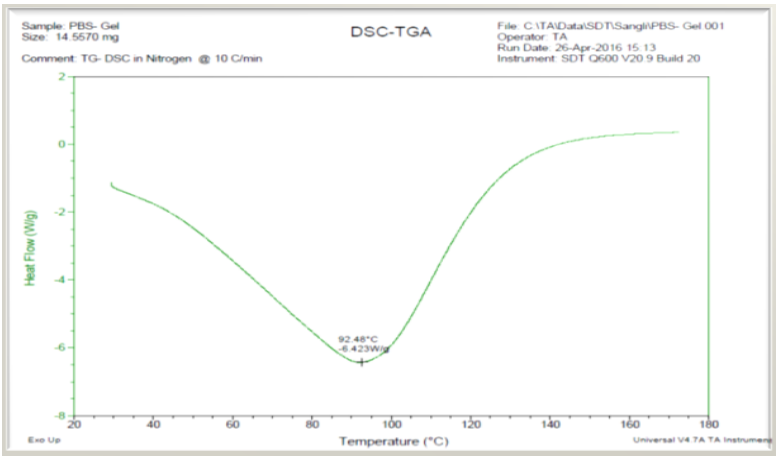

Figure 2: Differential scanning calorimetry curve

The analysis was performed by placing samples on a brass stub using a double-sided adhesive tape and was made electrically conductive by coating in vacuum (6 pas) with platinum using ah Ion Sputter at 15 mA. The microphotographs were taken at an excitation voltage of 10 kv. The SEM image show the relatively spherical (Figures 3 and 4).

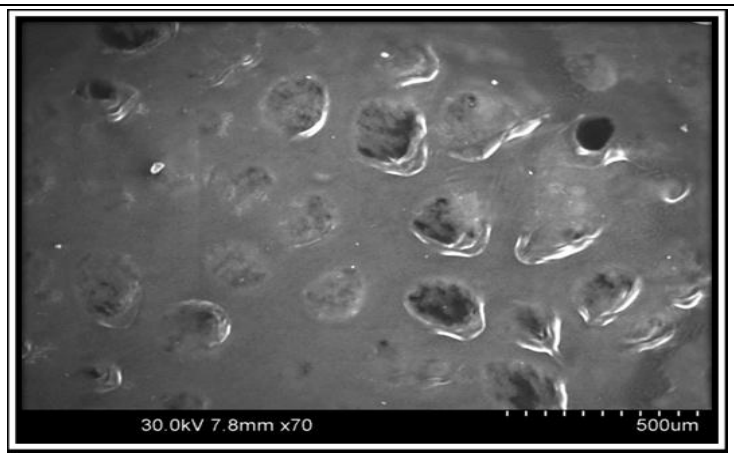

Figure 3: Scanning electron microscopy of formulated gel

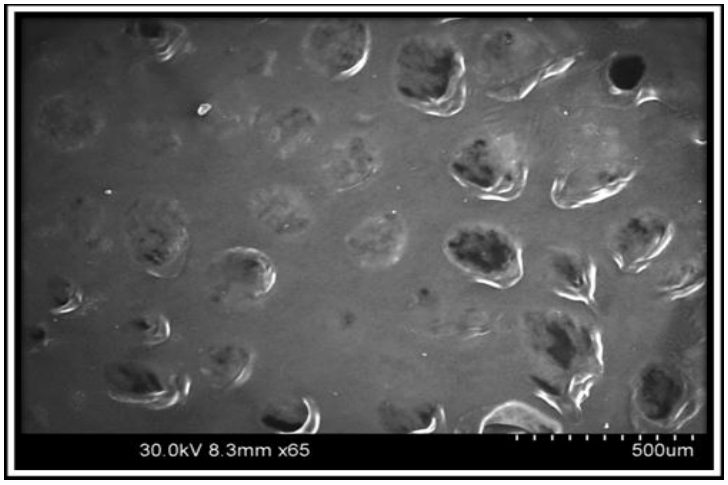

Figure 4: Scanning electron microscopy at 30.0 kV 8.3 mm × 65

***In vitro* method (Table 13 and Figure 5)**

Table 13: *In vitro* activity of formulation gel against HaCaT cell line

Compounds	Reading	% Inhibition	IC_{50}
Negative control	0.390		
Positive control (5 FU)	0.095	75.64	
10 µg/ml	0.066	83.07	
50 µg/ml	0.050	87.17	43.63 µg/ml
100 µg/ml	0.029	92.56	

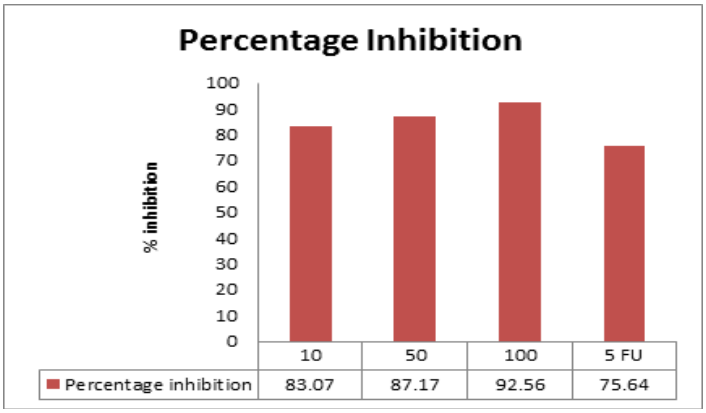

Figure 5: Percentage inhibition of samples using HaCaT cell line

Results: 100 µg/ml shows 92.56% inhibition with good antiproliferation activity compared to positive control

Perry scientific mouse tail method (Figures 6-8)

Figure 6: The length of granular layer in control group. (Under 10x) (L1=0.00166432 µm and L2=0.003042155 µm and Mean=0.002353 µm)

Figure 7: The length of granular layer in standard (Retino A 0.005% cream) group. Under 10x magnification (L1=0.004876474 µm and L2= 0.007889234 µm, Mean=0.006312 µm)

Figure 8: The length of granular layer in test group (formulated gel) (L1=0.002059126 µm and L2=0.002846050 µm, Mean=0.002452 µm)

DISCUSSION

The present study was aimed to investigate the antipsoriatic activity of gel containing methylsulphonylmethane Powder and seed oil of *P. pinnata* Linn. The physicochemical properties of gel formulation in which different batches (B1-B5) showed good appearance & homogeneity. The physical appearance of the gel formulation was white in nature. The pH of the gel formulations was determined by pH meter in which different batches (B1-B5) showed pH in range of 6.5-6.8 and the pH of optimized batch B2 was found to be 6.8. It lies in the normal pH range of skin and with time no skin irritation was observed. The viscosities of formulated gel were determined by using Brookfield Viscometer of different batches (B1-B5) and were ranging between 3782-5094 cps. The Viscosity of optimized batch B2 was found to be 4476 cps. Gels with high viscosity may not extrude from tubes whereas low viscous gels may flow quickly.

Hence suitable viscosity is required. Gel formulation majorly depends upon its viscosity. Viscosity of the formulation affects the drug release from the gel. If a gel consists of more viscosity the drug release from the formulation is decreased and if the same gel possess less viscosity the drug diffuses immediately into the diffusion medium. Hence for the gel formulation optimum viscosity is necessary to get the maximum drug release.

The values of optimized batch B2 (25.02 g.cm/sec) in spreadability indicates that gel was easily spreadable by small amount of shear as compared with other batches. It denotes the extent of area on which gel readily spreads on application to skin. Therapeutic efficacy of formulation depends upon spreadability of formulation. The extrusions of formulated gel of different batches (B1-B5) were determined and were showed in percentage in ranging between 82.65-95%. The extrusion of optimized batch B2 was found to be 92.45%. The extrusion of gel from tube is important during its application and in patient acceptance. Gels with high consistency may not extrude from tubes whereas low viscous gels may flow quickly. Hence suitable viscosity is required.

The calibration curve of MSM

The different concentration was prepared and their absorbance was taken at 470 nm. The percent drug content of all formulations was found to be in the range of 91.01-95.26% w/v. The difference in drug content may be due to human error during dilution or may be due to production loss during formulation.

***In vitro* diffusion study**

It was performed by using cellophane membrane of pore size 40 µm using Franz diffusion cell. Batch B2 shows 77.60% drug release. Generally it is said that cross-linking of polymers in formulation such as increases drug diffusion rate decreases. From observation it can be concluded that concentration of polymer increases drug diffusion rate was reduced. Thus diffusion is polymer concentration dependent phenomenon.

Skin irritation study

The skin Irritancy study indicated that formulated gel was free from dermatological action like signs of erythema and edema.

Stability study

Stability of pharmaceutical product is capability of a particular formulation in a specific container closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. Accelerated stability study was done for optimized Batch B2 for 3 months at 40°C and 75% relative humidity as per ICH guidelines. It indicates that that the physical appearance, pH, viscosity, extrudability, spreadability in the prepared gel remained unchanged upon storage for 3 months. The result of stability study indicates that formulation was stable at accelerated condition of temperature & relative humidity.

The IR spectrum

IR spectrum was carried out for the determination of functional groups in the sample. The optimized batch B2 of gel was characterized, the observation of principle peak was found at 1724 which indicates carbonyl group while Peak at 1149 indicates the presence of sulphonyl group.

DSC analysis of formulated gel

DSC curves of optimized Batch (B2) showed at 92.61°C which indicates possible change in melting point, release kinetics and bioavailability of drug.

SEM was used for determination

Surface topography, particle size, morphology of the gels. The SEM image have shown the relatively spherical and uniform particles of diameter 7.8 mm.

Pharmacological activity

In vitro activity of formulated gel of optimized batch B2 against HaCaT cell line

The present study aimed to investigate the antiproliferative properties of formulated gel. Antiproliferative effects against keratinocytes using cultured HaCaT cells as a psoriasis relevant experimental model. IC_{50} value was found to be 43.63 µg/ml.

Antipsoriatic activity by Perry's scientific mouse tail model

In vitro antipsoriatic activity of batch B2 showed the significant orthokeratosis in the mouse tail when compared to control thus indicating that the formulation is effective in treating psoriasis.

Aematoxylin-eosin staining act as an indicator of orthokeratosis. The granular layer is present in epidermis. It is greatly reduced or almost absent in epidermis of psoriatic lesions. This condition is known as parakeratosis, one of the most important characteristic features of psoriasis. The granular layer formation around the epidermis is known as orthokeratosis condition. The main principle behind the mouse tail test is conversion of parakeratosis to orthokeratosis. Morphometric measurement by motic microscope shows the thickness of granular layer around the epidermis is less in control group while moderate in standard group and highest in test group. Many herbs used in the treatment of psoriasis have been evaluated by this method, and were found to have significant effects. Psoriasis is a disease resulted from the hyper proliferation and abnormal differentiation of keratinocytes. A successful antipsoriatic drug that targets the epidermis is a compound that ideally shows low toxicity and restores skin homeostasis by suppressing keratinocytes hyper proliferation, abnormal differentiation, or both.

Substances like dithranol and vitamin D analogues that form keratinocytes differentiation are effective in bringing homeostasis of the epidermis in psoriasis conditions. The granular layer is greatly reduced or almost absent in epidermis of psoriatic lesions. The results of the histopathological study indicates that in control group, the nucleus was present in the keratinocytes of epidermis as well as the length of granular layer was found 0.002353 µm around epidermis. While in standard group and test group the nucleus was absent and the length of granular layer around the epidermis were 0.006312 µm and 0.002452 µm respectively.

CONCLUSION

Since psoriasis is a recurrent skin disease of multiple etiologies, we selected the drug of herbal origin to minimize the related side effects associated with synthetic drugs. The external application is be beneficial in the management of psoriasis. Topical formulations are widely accepted because it is having effective and easy administer. Our findings reveal that MSM and seed oil containing gel formulations is good in appearance, homogeneity, viscosity. Our study provides evidences that gel containing of seed oil of *P. pinnata* and methylsulphonylmethane combination shows antipsoriatic activity. *In vitro* antipsoriatic activity of batch B2 show the significant inhibition of proliferation of keratinocyte cell (HaCaT cell line).

However there is need for further investigations of antipsoriatic drugs to establish the clinical utility.

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my guide Dr. Sandeep, B. Patil, assistant professor of pharmacology, for his expert guidance and scholarly supervision, endless motivation immense knowledge, encouragements and freedom to carry research work. A sincere gratitude Dr. Mrs. N.S. Naikwade Head of Pharmacology Department for her expertise guidance and moral support every time i needed during my research work.

REFERENCES

[1] G. Misal, V. Dixit Gulkari, *Indian. J. Nat. Prod. Resource.*, **2012**, 3(4), 501-506.
[2] H.G. Vogel, Pharmacological Assays, **2002**, 2, 1323-1325.
[3] A. Vijayalakshmi, V. Ravichandiran, M. Velraj, S. Nirmala, S. Jayakumari, *Asian. Pac. J. Trop. Biomed*., **2012**, 2(4), 269-275.
[4] B. Bosman, T. Matthiesen, *Skin. Pharmacol*., **1992**, 5, 41-48.