FORMULATION AND EVALUATION OF HERBAL GEL FOR THE TREATMENT OF PSORIASIS

Mrinalini C. Damle¹, Mangesh R. Bhalekar*² and Sneha A. Lonkar¹

A.I.S.S.M.S. College of Pharmacy, Kennedy Road, Near R.T.O., Pune-411001, Maharashtra, India.

ABSTRACT
Psoriasis is regarded as an autoimmune disease in which genetic and environmental factors have a significant role. Natural remedies are more acceptable with the belief that they are safe and having less side effects. Herbal drugs have been used since many years not only in Asian countries but also worldwide for social well being. The present work deals with the formulation and evaluation of the herbal extract of root of Coleus forskohli (mainnula). In literature it is reported that this plant having antipsoriatic activity by regulating cAMP levels in skin cells to have therapeutic benefit for the sufferers of psoriasis. The composition of gel consist of Forskolin an active constitute, propylene glycol as a penetration enhancer and Sodium CMC as gelling agent showed the best results in final formulation. The percentage of drug release, viscosity & spreadiability profile of gel formulation was studied by $3^2$ factorial design. Accelerated Stability to check the physical appearance, rheological properties, drug content remains unchanged upon storage for 1, 2, 3 months at 40°C /75% RH condition. International Conference on Harmonisation Q8 guidelines was applied using $3^2$ factorial designs coupled with response surface methodology.

KEYWORDS: Psoriasis, Herbal, Coleus forskohli, Sodium CMC, Factorial design.

INTRODUCTION
Psoriasis is a chronic, inflammatory, multi-system disorder characterized by abnormal epidermal differentiation and hyperproliferation thought to be related to abnormal immune system activity. The name of the disease is derived from Greek word “psora”, which means “itch”. The T-cells, a type of white blood cell, become over-stimulated. They direct the skin
to try and “heal” a non-existent injury. The reaction of skin to psoriasis is the same as that to a fungal infection; it grows very fast, trying to “grow” the infection off the skin, new cells build up in the top layer of the skin. These areas become the reddened, inflamed, patches or plaques with white scale on them. Psoriasis is characterized by skin cells that multiply up to 10 times faster than normal.

Cyclic adenosine monophosphate (cAMP) regulates numerous key pathways that impact the immune system. They regulate the balance between synthesis by adenylyl cyclases and degradation by phosphodiesterases (PDEs). Distinct cellular cAMP signaling pathways can lead to both pro and anti-inflammatory effects depending upon the cell type. When dysregulated, these cAMP pathways can influence the pathogenesis of inflammatory cutaneous diseases. In psoriasis, cAMP and/or its effector proteins (e.g., protein kinase A) are downregulated. Pharmacologically inhibiting PDEs represents one effective mechanism to raise intracellular cAMP levels.

For treatment purposes, psoriasis can be categorized into localized and generalized forms, based upon body surface area (BSA) involvement. In any case, the treatment plan should include obtaining rapid control of the disease and maintaining that control. For generalized disease, systemic therapy approaches such as oral therapy, immunotherapy and UVB phototherapy are effective treatment options.

For localized mild to moderate disease, usually defined as lesions covering <10% of body surface area, topical therapy is often sufficient. However, it is not surprising that topical forms of therapy are more prescribed than systemic treatment. The topical therapy intends to improve quality of life with minimal adverse effects. However, this approach can decrease the number and thickness of the plaque lesions, and reduce the percentage of body surface involved. An overview of the topical antipsoriatic medications is summarized in Table 1.

Table 1: Summary of topical medication for psoriasis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids such as Betamethasone, clabetasol propionate</td>
<td>Ointment, gel, cream, foam</td>
</tr>
<tr>
<td>Vitamin D3 analogues such as Calcipotriol, Tacalcitol</td>
<td>Ointment, cream, solution</td>
</tr>
<tr>
<td>Coal tar, anthralin</td>
<td>Ointment, cream</td>
</tr>
<tr>
<td>Tacrolimus, picrolimus</td>
<td>Cream, ointment</td>
</tr>
<tr>
<td>Betamethasone dipropionate + salicylic acid</td>
<td>Ointment, cream, lotion</td>
</tr>
</tbody>
</table>
The term “Herbal drugs” denoted by means of plant or part of plants that have been converted into phyto pharmaceuticals. They include herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. Herbal treatments for the psoriasis are reported to be helpful in boosting up the immune system, give quick relief from symptoms and also prevent recurrent attack of psoriasis.

The Ayurvedic herb coleus (Coleus forskohlii) has been used historically as one of many herbal psoriasis remedies.[1] The genus Coleus forskohli (Lamiaceae) commonly known as Mainmula, is recorded in Ayurvedic Materia Medica under the Sanskrit name Makandi and Mayani.[3] Forskolin a major diterpenoid isolated from the roots of Coleus forskohlii, directly activates the enzyme adenylyl cyclase thereby increasing the intracellular level of cAMP and leading to various physiological effects.[7,8] The ability of Forskolin to regulate cAMP levels in skin cells has been shown to have therapeutic benefit for the sufferers of psoriasis.[2,5] The literature does not report any topical formulations of Forskolin for psoriasis, hence we have attempted to prepare and optimize Forskolin gel using Design of Experiment strategy.

MATERIALS AND METHODS

Material
Coleus forskohlii root purchased from Yucca Enterprises, Wadala (E), Mumbai. Other excipients such as Sodium carboxy methylcellulose, Propylene glycol, Propyl paraben and Methyl paraben were procured from Loba Chemie Ltd., Mumbai.

Methods
Preparation of herbal extract:[11]
C. forskohlii roots were collected washed and dried. After drying the tubers were pulverized. The pulverized granules (100g) were extracted with methanol (500ml) to obtain Forskolin. Methanolic extract was concentrated and 20 ml concentrated extract was partitioned between chloroform (60 ml) and water (60 ml) in a separating funnel. Allow to settle and separate the chloroform layer. Repeat the water treatment two to three times and collect chloroform layer, concentrated at 60°C till a semisolid mass was obtained. To the semisolid mass ice cold n-hexane was added to precipitate the Forskolin.
PREFORMULATION STUDIES

Fourier transformation infrared spectroscopy (FTIR) spectroscopy
The IR spectrum of Forskolin, Propylene glycol and polymer were recorded using Fourier transform infra-red spectrophotometer. Samples were prepared using KBr disc method and spectra were recorded over the range 400 cm\(^{-1}\) to 4000 cm\(^{-1}\). Spectra were analysed for drug excipients interactions and functional group involved in the formulation.

Determination of \(\lambda_{\text{max}}\) of Forskolin by UV spectroscopy
Methanolic solution equivalent to 1000µg/mL of calibration for Forskolin was constructed in phosphate buffer pH 6.8. Forskolin was diluted suitably in methanol to prepare dilutions of 10-50 mcg /ml, the absorbance was measured at 210nm and calibration curve was plotted to obey they Beer-Lamberts law. The equation of the line \(y=0.0037x + 0.0706\). \(R^2 = 0.9938\).

Preparation of the gel formulations\(^{[10]}\)
Propylene glycol were used as co-solvent for dried extract and also chosen as the best levigator. NaCMC was dispersed in preserved water (methyl paraben 0.18% and propyl paraben 0.02%) overnight. The extract was dissolved in propylene glycol and was added to the polymer dispersion and stirred with a double bladed mixer at 500 rpm for 10 min, until a homogenous gel was obtained. A 2 factor 3 level factorial design was used to obtain a formula with factor combination to give highest drug release, required viscosity and spreadability. The factors and values assigned to them are summarized in table 2. Nine experimental batches were prepared as per table 2 and evaluated for response parameters as described below.
Table 2: Formulae for preparation of gel formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Forskolin (g)</td>
<td>1</td>
</tr>
<tr>
<td>NaCMC (g)</td>
<td>2</td>
</tr>
<tr>
<td>Propylene glycol (ml)</td>
<td>10</td>
</tr>
<tr>
<td>Propyl paraben (g)</td>
<td>0.02</td>
</tr>
<tr>
<td>Methyl paraben (g)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Each formulation consists of preserved water (propyl paraben 0.02% w/w and methyl paraben 0.18% w/w) to 100 g.

EVALUATION OF FORMULATIONS

Viscosity
Brookfield digital viscometer (RVDV Pro), equipped with a T-bar spindle coded S-6 was used to determine viscosity (cp) of the formulations. The viscosity at measured at 100 rpm after 30 seconds.

Drug content evaluation
Content of Forskolin in formulation was determined by appropriately diluting the 1gm of the formulation with phosphate buffer pH 6.8. Absorbance was measured at 210 nm using uv visible spectrophotometer and % drug content was calculated.

Spreadability
Spreadability of formulation was determined using a Brookfield CT3 texture analyzer. The apparatus consist of two glass cone shaped probe. Fill one probe with 20 gm of gel (upto plane surface of top) and measure the spreadability. This apparatus shows spreadability into the comparison of hardness and adhesive force was calculated.

In-Vitro diffusion study
Franz diffusion cell was used for the drug release studies. The formulation was evenly applied on the surface of cellulose membrane. The cellulose membrane was clamped between the donor and receptor chamber of diffusion cell. The receptor compartment was filled with phosphate buffer pH 6.8, and the assembly was maintained at 37°C ± 0.5 under constant magnetic stirring. With reference to SUPAC guidelines laid by FDA, 1gm of formulation was applied to the membrane on the donor compartment and then covered with aluminium foil to prevent drying out. Aliquots were withdrawn at predetermined time intervals over a period of
1, 2, 3, 4, 5 and 6 hrs and analyzed spectroscopically.

**Optimization Data Analysis**
The outcome of experimental design were processed through design expert software (trial version 10.5). Statistical model were generated to predict the values of response variables at selected values of process variables within the design space. The model generated tested for statistical fitness using ANOVA. Optimized formulation was selected on the basis of values of desirability function. Formulation and The polynomial regression results were demonstrated using 3-D graphs and contour plots.

**RESULTS AND DISCUSSION**

**Preformulation studies**

**FTIR studies**
IR spectrum of Forskolin showed C-O Stretch at 1035 cm, O-H Stretch at 3412 cm, C-H Stretch at 1383 cm, C=C Stretch at 1624 cm.

![A](image1)

![B](image2)
Determination of $\lambda_{\text{max}}$ of Forskolin UV spectroscopy

The wavelength of maximum absorption ($\lambda_{\text{max}}$) was found to be 210nm which is same as reported value is referred. This wavelength was used for determination of drug content of formulation.

Preparation of the gel formulations
For the formulation of Forskolin herbal gel, NaCMC act as a viscosity modifier and water retention agent and it used in the rage of 2 – 4 gm. The second factor propylene glycol was used as penetration enhancer, in the concentration range of 10 – 20 gm.$^{[16]}$ Evaluation done by 3 responses, viscosity, spreadibility and % drug release. The $3^2$ factorial design were used for the optimization, where the number of variables under study tends to be lower and to evaluate the effects of each factors influencing the formulation based on statistical analysis.
Evaluation of gel formulations

Evaluation of all 9 batches are given in table 2 and fig. 3 shows cumulative amount of forskolin drug release (in %).

Table 3: Evaluation of Forskolin Gel

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Batches F1 F2 F3 F4 F5 F6 F7 F8 F9</td>
</tr>
<tr>
<td>Forskolin (g)</td>
<td>1 1 1 1 1 1 1 1 1</td>
</tr>
<tr>
<td>NaCMC (g)</td>
<td>2 3 4 2 3 4 2 3 4</td>
</tr>
<tr>
<td>Propylene glycol (ml)</td>
<td>10 10 10 15 15 15 20 20 20</td>
</tr>
<tr>
<td>Hardness (g)</td>
<td>31.6 52.2 80.7 51.26 44.5 81.17 76 63.8 70.9</td>
</tr>
<tr>
<td>In-vitro (%)</td>
<td>72.44 93.5 63.28 61.54 77.26 60.82 73.01 91.13 86.9</td>
</tr>
<tr>
<td>Viscosity (cp)</td>
<td>42 50 58 53 56 67 50 51.2 60</td>
</tr>
</tbody>
</table>

In vitro diffusion study: The gel were evaluated for in-vitro diffusion profile.

Fig. 4: Cumulative amount of Forskolin drug release (in %) from gel formulation through cellulose membrane using Franz diffusion cell.

Optimization by using $3^2$ factorial design

Analysis of experimental results

Response data of all formulations were fitted to model. Best fitted model was quadratic for all responses. Further, analysis using ANOVA indicated significant effect of the formulation factor on response parameters.

Mathematical model

The $r^2$ of hardness, viscosity and % drug release was found to be 0.9464, 0.9827 and 0.9410 respectively. The F value of hardness, viscosity and % drug release was found to be 10.58, 33.99 and 9.57 respectively. The P value of hardness, viscosity and % drug release was found to be 0.0402, 0.0076 and 0.0461 respectively.
F value for models was found to be high which indicated that the models were significant. P value less than 0.05 indicated that the model terms were significant. High $R^2$ values indicated good agreement between formulation variables and response parameters. Thus the models can be used to predict the values of the response parameters at selected values of formulation variables within the design space.

**Response surface [3D] and counter plot**

The relationship between formulation and response variable was studied by constructing counter and 3D response plots. The effect of concentration of propylene glycol and NaCMC are shown in figures as follows:

1. **Hardness:** Hardness is a term which is inversely related to spreadability.

   ![Response surface for Hardness](image)

   **Fig. 5: Response surface for Hardness**

   Equation of hardness: $+ 51.13 + 12.32 \times A + 7.70 \times B - 13.55 \times AB + 11.77 \times A^2 + 3.56 \times B^2$

   The factor A i.e. NaCMC has a positive effect hence as the concentration of NaCMC increases the hardness increases whereas as factor B too has positive effect hence, as the concentration of propylene glycol increases the hardness also increases. Thus, spreadability which reduces with increasing hardness is affected adversely by NaCMC and propylene glycol both.
2. Viscosity

![Fig. 6: Response surface for Viscosity](image)

Equation of Viscosity = + 56.93+6.67*A+1.87*B-1.50*AB+2.60*A²-6.80*B²

The factor A i.e. NaCMC has positive effect hence, as the concentration of NaCMC increases the viscosity increases whereas factor B propylene also has positive effect. Hence, as the concentration of propylene glycol increases the viscosity also increases.

3. % Drug release

![Fig. 7: Response surface for % drug release](image)

Equation of % Drug Release = + 78.29+0.67* A+3.64* B+5.76* AB–17.63 * A²+13.50* B²

The factor A i.e. NaCMC has a positive effect on drug release while PEG too shows same effect. At high concentrations NaCMC decreases the release due to very high viscosity. Similarly factor B also has positive effect on release due to its ability to draw water but has
detrimental effect at high concentration. The NaCMC shows higher and continuous increase in release at high concentrations of PEG.

**Optimization and formulation**

The formulations prepared as per the experimental design were evaluated and the analysis of experimental results was done by using the Design Expert.

**Table: 4. Evaluation of formulation**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Hardness (g)</th>
<th>In-vitro (%)</th>
<th>Viscosity (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td>62.38</td>
<td>92.43</td>
<td>52.0</td>
</tr>
<tr>
<td>Observed</td>
<td>63.8</td>
<td>91.13</td>
<td>51.2</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Natural remedies are safe as well as having less side effects. In the world market, herbal formulations are in a great demand. It is a very good attempt to establish the herbal gel containing extracts of Coleus forskohli root. This study concludes that the developed formulation of batch F8 was comparatively better than other formulations.

**ACKNOWLEDGEMENT**

The authors would like to thank A.I.S.S.M.S. College of Pharmacy, Pune. Maharashtra, India for providing necessary facilities to carry out the research work.

**REFERENCES**


13. Levy J, Zhou DM, Zippin JH. Cyclic Adenosine Monophosphate Signaling in Inflammatory Skin Disease; Clinical & Experimental Dermatology, 2016; 7(1).

