# *Chapter 10*
# *Summary & Conclusion*

Steroid-Responsive Dermatoses, or SRD, are a group of skin conditions that respond to treatment with topical corticosteroids. These conditions are diverse and include—plaque psoriasis, eczema, atopic and contact dermatitis, pityriasis rosea etc. Atopic dermatitis is one of the common steroid responsive dermatoses affecting over 10% of children, and it is the most common cause of occupational disability in adults.

Atopic dermatitis (AD) is a chronic allergic inflammatory disease which manifests itself as eczematous skin lesions. The clinical phenotype of atopic dermatitis is the product of interactions between susceptibility genes, the environment, defective skin barrier function, and immunologic responses. Although the precise mechanism underlying atopic dermatitis has remained unclear, it appears that immediate Immunoglobulin E IgE -mediated mast cell type , late IgE-mediated Th2 type and delayed IgE- independent Th1 type allergic reactions are involved in atopic dermatitis. Immunological analyses of the pathogenesis of atopic dermatitis have revealed that activated mast cells and eosinophils and an excess of differentiated T-helper (Th) 2 cells might play important roles in the development of dermatitis. Both Th cell subsets may contribute to the pathology of atopic dermatitis. Atopic dermatitis is a chronic and relapsing inflammatory skin disease characterized by episodes of intense pruritus, multiple lesions with erythema, excoriation, erosions, lichenification, papules, dry skin, and susceptibility to cutaneous infection. A major factor that exacerbates AD is xerosis.

Topical corticosteroids remain one of the most important treatments available for AD. Topical corticosteroids are available in a variety of vehicles— creams, ointments, lotions, gels, and more recently, foam. The vehicle used can substantially affect the individual agent's clinical action, potency, and acceptability to the patient. Moreover, some vehicles are better suited for

specific body areas. Patients' irrational fear about using topical corticosteroids has become a major barrier to effective long term management of severe AD.

The corticosteroids have a multiplicity of actions; anti-inflammatory, immunomodulatory, vasoconstrictor, gluconeogenic, anti-mitotic to name a few. It is believed that several of these actions contribute to the therapeutic efficacy of these drugs in the treatment of skin disease. Indeed it is often this multi-pronged attack that endows the Corticosteroids with the considerably greater therapeutic potency above other modes of treatment. The major anti-inflammatory effects are mediated through suppression of transcription of various genes encoding proinflammatory proteins. Besides, they also activate production of anti-inflammatory Lipocortin-1. Corticosteroids also exert their antiproliferative effects on T cells, fibroblasts, eosinophils etc.

Topical corticosteroids are associated with various side effects that may limit their use. These include localized skin reactions occurring at the site of application and generalized adverse effects from systemic absorption of corticosteroid. Local cutaneous reactions are more common than systemic side effects and are largely due to the antiproliferative effects of these agents. They include atrophy or thinning of the skin, striae, telangiectases, acneiform eruption, rosacea, and contact dermatitis. Systemic side effects, although uncommon, may occur when locally applied corticosteroids become absorbed through the skin and enter the general circulatory system and includes the suppression of Hypothalamus pituitary adrenal axis.

Halobetasol propionate is an ultra potent corticosteroid available as ointment and cream for clinical use, out of which ointment being occlusive is more potent. Being an ultrapotent steroid, a potential for serious local and systemic side effects is associated with its use.

Topical calcineurin inhibitors (tacrolimus and pimecrolimus) are recommended as second-line treatment for moderate-to-severe atopic eczema not controlled by topical corticosteroids, or when there is a high risk of adverse effects such as skin atrophy.

Tacrolimus acts directly on the T-lymphocytes, especially CD4+ cells, by binding to the FK-binding protein (FKBP). This tacrolimus-FKBP complex then binds to and competitively inhibits calcineurin which in turn inhibits transcription factor, NFAT (nuclear factor of activated T-cells). It is this transcription factor that activates the promoter region of the gene for various inflammatory cytokines that participate in the early immune response and are postulated to play a role in AD pathogenesis. Tacrolimus inhibits the release of mast cell and basophil preformed mediators, downregulates IL-8 receptor expression, and decreases chemokines. This broad range of the inflammatory inhibition mechanism may reduce antigen recognition and downregulate the entire inflammatory cascade leading to clinical disease. The main side-effects are skin irritation, burning, erythema, infections and alcohol intolerance.

Human skin is an important target site for the application of drugs. Especially in the treatment of local diseases, a topical drug delivery is an appropriate strategy to restrict the therapeutic effect on the affected area and to reduce systemic incrimination. Topical delivery is affected by various factors, e.g., the physicochemical properties of the drug and the vehicle used for application, condition of the epidermal barrier etc.

Methods for improving cutaneous delivery rely on the use of chemical penetration enhancers, novel vehicle systems (e.g., microemulsions, liposomal-based delivery systems and supersaturated formulations), or more

complex physical enhancement strategies (e.g., iontophoresis , sonophoresis and electroporation).

Modern drug carrier systems microemulsions (MEs) are thermodynamically stable, low viscous, transparent and optical isotropic formulations with a dynamic microstructure that form spontaneously by combining appropriate amounts of a lipophilic and a hydrophilic ingredient, as well as a surfactant and a co-surfactant. A number of investigations have been carried out which demonstrated that drugs incorporated into microemulsions penetrate efficiently into the skin and through the SC-barrier.

There are several permeation enhancement mechanisms of microemulsions such as an increased concentration gradient and thermodynamic activity toward skin and the permeation enhancement activity of the components of microemulsions. Important features of ME are their high drug solubilization capacity, which leads to high concentration gradients towards the skin and a microstructure that allows free and fast drug diffusion. Having low or no interfacial tension, MEs are thought to rapidly penetrate into the stratum corneum where they will blend into skins mantle. They are relatively stable and can solubilize a considerable amount of hydrophobic drugs in their lipophilic domain. The mechanism of enhancement via drug supersaturation is based simply on the increased thermodynamic activity of the drug in the vehicle, that is, an increased driving force for transiting out of the formulation and going into and through stratum corneum.

More specific approaches to minimize side effects associated with topical corticosteroids include combination. The rationale assumes that agents are selected on the basis of their individual mechanisms of action, which may offer the possibility of one or more of the following: (1) additive or synergistic

efficacy, (2) reduction in the dosage of either or both products, and (3) reduction in the occurrence of side effects.

The present studies focuses on development and characterization of microemulsion based topical creams of a corticosteroid and calcineurin inhibitor. It is also aimed to develop a microemulsion based cream for the combination of two drugs.

The microemulsion based combination cream will enhance dermal penetration of the individual agents and thus it will be possible to achieve equivalent therapeutic effect with lower dose of the drugs. The present work will develop an aqueous cream giving higher penetration and will be having higher patient acceptability.

A combination of topical corticosteroid and calcineurin inhibitor is not available commercially, although the two agents have different mechanism of action, are each others alternative and have not been reported to be incompatible chemically. Since the two drugs are have non-overlapping mechanism of action and corticosteroid can control the side effects produced by calcineurin inhibitor, it is expected to achieve a synergistic effect and with less side effects.

The developed spectroscopic determination methods of HP were based on the zero order UV spectra giving maxima at 239 nm in methanol. It was found to be linear in the range of 5-45 μg/mL and had high accuracy and precision. A colorimetric method based on the reaction of $\alpha$ – ketols with tetrazolium blue dye under basic conditions was the basis for estimation of HP in formulations. It was linear in the range of 2.5-15 μg/mL and demonstrated reasonable accuracy and precision.

The developed chromatographic method of HP in diffusion media, skin extracts and formulations was based on reversed-phase HPLC method with UV detection at 239 nm. The mobile phase comprised acetonitrile: methanol (55:45 v/v) at a flow rate 1.0 $mL \cdot min^{-1}$. The calibration plot was linear in the concentration range of 0.1-20 μg/mL and had high accuracy, precision and fulfilled the system suitability requirements.

Tac showed a zero-order peak at 210 nm in methanol hence, a simple UV spectroscopic method was not employed for its estimation. The developed chromatographic method of Tac in diffusion media, skin extracts and formulations was based on reversed-phase HPLC method with UV detection at 210 nm. The mobile phase comprised methanol: water (90:10) at a flow rate of 0.8 $mL \cdot min^{-1}$. The calibration plot was linear in the concentration range of 10-250 μg/mL and had high accuracy, precision and fulfilled the system suitability requirements.

Another reversed-phase HPLC method with UV detection at 210 nm was developed for Tac so that it could be estimated in combination cream with ease. It had the same mobile phase as HP i.e. acetonitrile: methanol (55:45 v/v) at a flow rate 1.0 $mL \cdot min^{-1}$. The method was linear, had high accuracy, precision and fulfilled the system suitability requirements.

Solubility studies on the drugs were carried out to find oils, surfactants and co-surfactants which will dissolve higher quantities of drug. It was found that HP exhibited higher solubilities in capmul MCM L8, isopropyl myristate, Tween 80®, Transcutol P®, Soluphor P®, PEG 200. Similarly Tac exhibited higher solubility in capmul MCM C8, ethyl oleate, Tween 80, Soluphor P, Transcutol P and PEG 200.

Based on the results of solubility studies, three systems were explored for ME preparation of HP. For HP, 3 systems were prepared which are System 1 [Capmul MCM L8, Tween 80 + Transcutol P (1:1), Distilled water] and System 2 [Isopropyl Myristate, Tween 80 + Transcutol P (2:1), Distilled water] and System 3 (Capmul MCM L8, {Tween 80 + (Transcutol P: PEG 200(1:1)) (2:1)}, Distilled water]. The ratios of the constituents were optimized by constructing pseudo ternary phase diagrams and $3^2$ factorial design. The independent variables for factorial design were oil concentration and surfactant concentration while the dependent variables were globule size and zeta potential. Contour plots were developed for each system and using the predicted and experimental data, one batch from each system was selected for further characterization. The selected batches were evaluated for drug loading, viscosity, refractive index, assay etc. and one optimized batch was selected for HP. The optimized batch had a constitution of capmul MCM L8 (3.75 %) as oil phase, Tween 80 (15 %) as surfactant and Transcutol P (15 %) as co-surfactant.

Based on the results of solubility studies, three systems were explored for ME preparation of Tac. For Tac, 3 systems were prepared which are System 1 [Capmul MCM C8, Tween 80 + Transcutol P (1:1), Distilled water] and System 2 [Capmul MCM C8, Tween 80 + Soluphor P (1:1), Distilled water] and System 3 (Ethyl oleate, Tween 80 + Transcutol P (2:1), Distilled water]. The ratios of the constituents were optimized by constructing pseudo ternary phase diagrams and $3^2$ factorial design. The independent variables for factorial design were oil concentration and surfactant concentration while the dependent variables were globule size and zeta potential. Contour plots were developed for each system and using the predicted and experimental data, one batch from each system was selected for further characterization. The selected batches were evaluated for drug loading, viscosity, refractive index,

assay etc. and one optimized batch was selected for Tac. The optimized batch had a constitution of capmul MCM C8 (3.75 %) as oil phase, Tween 80 (15 %) as surfactant and Transcutol P (15 %) as co-surfactant.

The drug loaded microemulsions were incorporated in cetomacrogol cream base by replacing an equivalent quantity of water. The final concentrations for HP were 0.05% (clinical dose) and 0.035% (lower dose) and 0.1% Tac (clinical dose) in the cream. The drug loaded creams were also characterized for physical appearance, assay, viscosity, pH.

Transmission electron microscopy was carried out for drug loaded microemulsion and ME based creams. The microemulsions were seen after direct drying on copper grid and it was observed that the average globule size was less than 50 nm and corresponded to dynamic light scattering results. Cetomacrogol cream base was also visualized before and after incorporation of ME, by TEM with negative staining. It was observed that number of globules below 100 nm increased after incorporation of ME into cetomacrogol cream base. This indicates that ME retains its microstructure after incorporation into semi solid base.

Accelerated stability studies and long term stability studies were carried out on 2 optimized ME and cream formulations of HP and Tac. It was observed that MEs for both drugs were found to be stable in accelerated studies. The ME and cream formulations were also stable for 6 months at RT and 2-8°C with no loss in potency.

The drug loaded formulations were further characterized by in-vitro drug release studies through semi-permeable cellulose acetate membrane and skin retention and permeation through rat skin and human cadaver skin. HP

formulations [HP solution in propylene glycol, HPME, HPMEC 0.035% and 0.05%, HP cream and ointment (marketed preparations)] were subjected to *in vitro* diffusion studies. The kinetic pattern of the diffusion was studied by fitting % drug diffused in given time in different order kinetics and the release pattern of HP from the formulations followed Higuchi kinetics. Solution and drug loaded ME showed near complete release in the first hour. The ME based cream showed slightly faster drug release in comparison to the commercial cream. The ointment demonstrated a significantly slower and incomplete release in 24h. In ex vivo studies with rat skin and human cadaver skin, HP could not be detected in receptor solution even after 24 h of application. The drug retention in skin followed the rank order - HP Solution > HP ME > HP MEC (0.05%) > HP ointment ~ HP MEC (0.035%) > HP cream.

Tac formulations [Tac solution in propylene glycol, Tac ME, Tac ME based cream 0.1% and Tac ointment (marketed preparation)] were subjected to *in vitro* diffusion studies. The kinetic pattern of the diffusion was studied by fitting % drug diffused in given time in different order kinetics and the release pattern of Tac from the formulations also followed Higuchi kinetics. Solution and drug loaded ME showed near complete release in the first hour. The ME based cream showed slightly faster drug release in comparison to the commercial cream. The ointment demonstrated a significantly slower and incomplete release in 24h. In ex vivo studies with rat skin and human cadaver skin, Tac could not be detected in receptor solution even after 24 h of application. The drug retention in skin followed rank order: solution > ME > MEC > ointment for both biological membranes. Drug being present in a supersaturated condition in MEs enhance dermal penetration due to high thermodynamic activity of the drug. MEs alter solvent properties of stratum corneum to favor drug partitioning into the skin. The lipophillic drug is

retained in lipidic skin layers and thus, drug is not detectable in receptor solution.

A sub-acute toxicity study of the formulations was carried out on wistar rats for duration for 2 weeks. The animals were divided into 4 groups each containing six animals, viz. control (Placebo), HPMEC 0.035% and 0.05% and TacMEC 0.1%. At the end of 2 week study, blood was removed from the animals and they were sacrificed and the excised skin from the site of application was removed and histopathology was performed. It was observed during the study that the animals did not exhibit any signs of local toxicity (edema, erythema or eschar formation) at the site of application in all the four groups. The blood indices did not exhibit any significant difference between the groups. The Hematoxylin–eosin stain of the sections of the excised tissue also did not demonstrate any epidermal toxicity or subepidermal signs of inflammation, edema etc. for all the 4 groups. The number of mast cells also reflected the same trend, with no significant difference between any of the groups.

The extent of skin irritation of the mouse skin was evaluated by MTT conversion assay on excised tissue. It can be seen from the optical density and relative viability data that all the developed formulations are relatively non toxic and non-irritant. The tissue undergoes severe necrosis when treated with positive control.

Acceptability testing in humans was carried out for 48 h, the placebo cream scored lower than HPMEC 0.035%, HPMEC 0.05% and TacMEC 0.1%. However, there was no statistical significance indicating that there was no significant difference between the four products tested at either assessment at 24 h and 48 h

The qualitative aspects of drug penetration in skin was also compared using fluoroscent microscopy. 6- coumarin loaded formulations were compared after 6 h of application on mice skin. It is evident that ME based cream ensured higher and deeper penetration in comparison to the marketed cream and ointment

The efficacy and safety of the formulations was evaluated in hapten (TNCB) induced model of dermatitis. The repeated application (sensitization and elicitation) of TNCB, results in development of an antigen-specific hypersensitivity response which is mix of immediate type hypersensitivity and delayed type response. These reactions are also characteristic of clinical cases of AD.

In murine model, a comprehensive dermatitis score can be calculated from the observed severity of 4 major symptoms of lesional skin. At the end of 2 week treatment, the reduction in dermatitis score with low dose HP ME based cream is equivalent to commercial cream and ointment both. It was observed that dermatitis score does not reduce significantly with HP ME based cream (0.05%). Skin atrophy is seen at the site of application with HP ME based cream (0.05%). HP ME based cream in clinical strength did not reduce the dermatitis score as it showed skin atrophy and associated erythema after 2 weeks of treatment. This may be attributed to the increased drug concentration in skin with microemulsion based cream. Skin atrophy is a common local dose dependent side effect. Tacrolimus application improves the morphological changes significantly from the very first week. The reduction in dermatitis score is higher with ME based cream within 7 days of application. At the end of 2 week treatment also, a significantly better control over symptoms is observed with novel formulation.

The anti-inflammatory responses are characterized by ear swelling studies in murine models as it is considered as a very reliable parameter for evaluation of therapeutics. The measurements are made 3h and 24h post application so as to study the effects on early and late phases of hypersensitivity response. The anti-inflammatory response (at 3h and 24h) is comparable between the HP ME based creams (0.035% and 0.05%) and commercially available cream and ointment at the end of 2 weeks treatment. Noteworthy is the equivalent anti-inflammatory response with low dose HP ME based cream due to enhanced permeation and skin retention of drug. The anti-inflammatory response is comparable between the Tac ME based cream and commercially available ointment at the end of 2 weeks treatment.

It is observed that total IgE levels are not suppressed with HP application. There is no significant difference between any of the groups. It was observed that total IgE levels were not suppressed with Tacrolimus application. There is no significant difference between any of the groups.

The histopathology of skin biopsy specimens shows that epidermal hyperplasia, spongiosis and increased dermal infiltrate of inflammatory cells and mast cells is seen after chronic application of hapten. A significant reduction of epidermal thickness with all HP formulations is seen. The protective effects are with low dose ME based cream is comparable to other formulations. Epidermal thinning is more prominent for ME based cream (0.05%). The reduction in epidermal hyperplasia is more prominent with the Tac ME cream as compared to ointment. However, the number of mast cells does not reduce with any of the formulations. The histopathological observations show that tacrolimus application restores the epidermal thickness, reduces the dermal infiltration of inflammatory cells.

Total RNA was isolated from excised ears at various time points during the course of study and Th1 (IL-2 and IFN-gamma) and Th2 (IL-4, IL-10) cytokine gene expression was assessed by RT-PCR as AD is thought to be a mixed Th1 and Th2 response. An equivalent concentration of total RNA isolated from mice ears was used for cDNA preparation and consequent PCR amplification using gene specific (IL-2, IL-4, IL-10 and IFN-gamma) primers. It was observed that at the end of elicitation phase, the expression of all the cytokines was significantly higher in comparison to basal level at the start of study. The increased expression of IL-4 and IL -10 in comparison to IL-2 and IFN-gamma on chronic application demonstrates a Th2 dominant response. After treatment with vehicle control (placebo) and drug loaded formulations, a significant reduction of cytokine expression was observed with HP formulations. IL-4 shows a marked reduction in the HP treated groups, although the levels do not drop to basal level after 2 weeks of treatment with HP cream (marketed preparation). IL-10 is also significantly upregulated in the challenged group and almost similar upregulation is observed in all the HP treated groups. In case of IL-2, there is a significant reduction with all formulations. IFN-g remains undetected in treatment groups. Vehicle control does not have any significant impact on cytokine expression.

The ME based cream suppresses IL-4 expression significantly more than the ointment. IL-10 is also significantly upregulated in the challenged group and almost similar reduction is observed in all the tacrolimus treated groups. In case of IL-2, the ME based cream restores the cytokine expression to basal levels. There was a significant reduction with the ointment also, but to a lower extent. The expression level of IFN-gamma was below detection at basal level and showed a rise after elicitation. It remains undetected in treatment groups. Thus, a combined suppressive effect on multiple cytokine expression by

microemulsion based creams translates into therapeutic benefit for better control of AD.

The vasoconstrictor assay or the skin blanching bioassay study was carried out for HPMEC 0.035% in comparison to HP cream 0.05% in healthy human subjects. The results show an improvement in efficacy of the product when formulated as microemulsion based cream. The pharmacodynamic response was seen to elicit faster in case of the nanotechnology based product. It was observed that the skin blanching lasted longer, suggesting an increased permeation and retention of the drug and hence, enhanced therapeutic efficacy of the product thus giving an equivalent response even in lower dose.

A pilot clinical study was carried out in dermatitis patients. The patients were divided into two groups. Group 1 was Microemulsion based halobetasol propionate cream (0.035%) and Group 2 was Plain halobetasol propionate cream (0.05%). Right –Left strategy was used for placebo application to minimize biological variations. The disease severity score based on the clinical course, disease intensity and extent of body surface involved was statistically similar in the two groups viz. microemulsion based cream 0.035% and plain cream 0.05% group. The scores indicated a moderate to severe progression of disease in the patient groups. There was almost similar reduction in erythema .with both the products, achieving near complete control over symptoms. The reduction in excoriation was also not statistically significant between the 2 groups, but values indicated a better therapeutic response with ME based cream. It was observed that placeboes don't have any significant impact on alleviating excoriation and the medicated preparations are more effective. The % reduction of scaling at the end of study was higher with the microemulsion based cream as compared to plain cream. The % reduction of pruritus at the end of study was significantly higher with the microemulsion based cream as

compared to plain cream and significantly higher from their respective placebos. The reduction in lichenification in both groups was significant only after the second week when the active treatment started. The % reduction of lichenification at the end of study in ME cream group was significantly more than Plain cream group and significantly higher from their respective placebos. The patient's global assessment of the treatment revealed that more number of patients experienced better control over disease with the use of HPMEC 0.035% as compared to HP cream 0.05%. The patients and physicians both reported higher number of patients with grade I improvement in global assessment. Thus these studies clearly demonstrate the effectiveness of low dose HPMEC 0.035% over HP cream 0.05% in dermatitis patients and presents a scope for dose reduction of a high potency corticosteroid.

An attempt was made to investigate a combination cream of HP and Tac. Although, several researchers have advocated the use of combination in treatment of dermatitis, such a combination is not available commercially. In fact, no chemical incompatibility between corticosteroids and tacrolimus has been reported. Clinically a combination in single formulation is not used but often sequential treatment with these two drugs is employed where tacrolimus is applied at night and corticosteroid in the day time. A fixed dose combination ME based cream of HP and Tac was developed wherein the concentration of HP is 0.035% and Tac is 0.1%.

An accelerated drug – drug compatibility study was carried out to explore any potential chemical or physical incompatibility between the two drugs. . It was found that mixture does not exhibit any change in color or other physical attributes. DSC exhibits a shift in the melting peak of HP but, the shift is seen at day 0 also. This can be interpreted as a shift in melting point due to presence of another low melting compound. Chemical compatibility was

further confirmed by HPLC where no extra peaks or significant change in area of peaks were observed.

The MEs for both the drugs were prepared according to optimized formulae and were incorporated into cetomacrogol cream base by replacing an equivalent quantity of aqueous phase. It was found that the combination was stable for at least 3 months with no loss in potency.

An *in-vitro* drug release study through semi-permeable cellulose acetate membrane was carried out. The drug release follows a very similar pattern and shows no significant change in comparison to their individual ME based creams. *Ex-vivo* drug diffusion and skin retention studies were also done through rat skin and human cadaver skin. It was found that the both the drugs exhibited slightly higher drug deposition in skin in comparison to individual cream.

Pharmacological evaluation of the combination was carried out in hapten induced mice model of dermatitis. It was found that there is no statistical significant difference in the dermatitis score. The individual ME creams, combination ME cream and sequential treatment showed almost similar therapeutic response. The combination treatment showed an elevated serum IgE levels in comparison to other treatment modalities. When ear swelling response was characterized at 3 h and 24 h post application it was found that a more pronounced decrease in ear swelling was observed on the first day of therapy with combination cream than individual ME cream. But, there is no significant difference between combination ME cream and sequential treatment. Cytokine gene expression was evaluated by RT-PCR technique. IL-10 was more elevated with combination ME cream and after sequential treatment as compared to individual ME creams. IL-2 expression is also

suppressed with combination and sequential treatment and levels go below the basal levels after 2 weeks of treatment. . It was observed that the corticosteroid containing formulations were more effective in suppressing inflammatory cytokine like IL-4. However, there was no significant difference between combination ME cream and sequential treatment.

To conclude, microemulsion based creams of halobetasol propionate and tacrolimus individually and in combination were successfully prepared, optimized, characterized and assessed pharmacodynamically in hapten induced mice model of dermatitis. Halobetasol propionate formulations were also evaluated clinically in healthy human subjects by skin blanching and in dermatitis patients. The clinical evaluation of tacrolimus cream could not be performed due to agreement with funding agency. However, the studies in this investigation demonstrate the permeation enhancement capabilities of microemulsion based creams and equivalent efficacy in comparison to occlusive ointments. Topical diseases, such as AD, require prolonged medication and complete remission of the disease is not often possible due to insufficient drug localization in the targeted layers of the skin (viable epidermis and dermis). But with the use of microemulsion based cream, high potency corticosteroid was equally effective in around 30% lower dose both in mice model and clinically. Cytokine gene expression studies in animal model further validated the efficacy of developed formulations of both drugs on molecular level. However, the developed combination microemulsion based cream did not demonstrate any significant benefit over sequential treatment. But, a combination may prove to be better choice of treatment for immediate control of severe cases of AD. The advantage would be reflected in better control of side effects of the individual drug. The burning sensations and pruritus associated with tacrolimus would be effectively controlled by corticosteroid and tacrolimus does not show atrophic effects like

corticosteroids. The developed formulations are likely to alleviate drawbacks of current management of disease and provide a patient friendly aqueous based dosage form of drugs. The enhanced permeation can also be translated to possibility of dose reduction. However, the therapeutic benefits need to be further confirmed by toxicology and more elaborate clinical studies.