RESEARCH ARTICLE

Teriflunomide Transdermal Cream: *In-vitro* And *In-vivo* Evaluation for the Treatment of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid Arthritis (RA) is an incurable long-term disease that requires continuous safe treatment. Currently, many anti-inflammatory agents and steroids are used to manage the diseases and are associated with a variety of side effects. There is a great need for a formulation that addresses the root cause of the disease and can be used for prolonged periods without any side effects. Hence, an attempt was made to develop a formulation of an alternative to steroid formulations that can be used for prolonged periods without/minimal side effects, i.e., teriflunomide topical dosage form. Teriflunomide tablet was approved for multiple sclerosis. It is the primary metabolite of leflunomide which is approved to treat RA. When leflunomide is administered orally converts to teriflunomide to elicit its action as such, leflunomide has no anti-rheumatoid effect. Both leflunomide and teriflunomide have different GIT side effects such as diarrhea, nausea, and hepatotoxicity on oral administration. To avoid these oral side effects of leflunomide and teriflunomide and to improve RA treatment compliance, teriflunomide was formulated as a topical cream. The developed topical cream is designed to be applied at night on the affected joints. It is intended to release the drug during the night, provide higher localized concentrations of the drug in the synovial joint and reduce GI and systemic side effects improving treatment compliance.

Keywords: Teriflunomide, Rheumatoid arthritis, Topical cream, Synovial joint, Drug targeting.

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INTRODUCTION

Rheumatism has an impact on the tendons, ligaments, and muscles around the joints. It can manifest in a variety of ways, depending on the patient's age, location, severity, and symptoms.[1] It causes inflammation and, damages joint cartilage and leads to deformity and irreversible chronic impairment. This disease usually affects the knees and shoulders.[2,3] Systemic and local corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and/ or physical therapy are the current choice of treatments for rheumatoid arthritis. The choice of therapies includes the use of NSAIDs, corticosteroids (Systemic or local), and/or physical therapy.[4]

The delivery of pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin (IL-1), etc, into the synovial cavity triggers the inflammatory response in rheumatism. As a result of their release, vascular cellular infiltration, hepatocyte protein C synthesis, and osteoclast activity with bone erosions will increase. These defensive reactions result in tissue damage that gets worse with time, lowering quality of life by causing joint degradation, functional impairment, and discomfort.[5] NSAIDs, such as acetic acid derivatives (diclofenac), propionic acid derivatives (ketoprofen), and salicylates (aspirin), etc are used in pharmacotherapy to modulate or lessen the inflammatory process by preventing the body from producing prostaglandins. The COX-2 (cyclooxygenase) enzyme is inhibited to stop the generation of 'harmful' prostaglandins.[6,7] Since topical NSAIDs have fewer systemic adverse effects than oral NSAIDs, they are becoming the drugs of choice to treat the joint by providing at least comparable analgesia, enhanced bodily function, and decreased stiffness.[8]

A number of plant species can produce methyl salicylate (also known as methyl esters of salicylic acid), which is an organic volatile molecule.[9,10] It is a rubefacient with a topical analgesic action and promotes blood circulation.[10] There are several topical formulations that combine methyl salicylate and diclofenac sodium for the management and/or treatment of RA. These drugs help to enhance joint flexibility and mobility by reducing pain, edema, and joint stiffness.[11]

The relationship between oxidative stress and persistent inflammation in rheumatism is well-known.[12] Reactive oxygen species (ROS) have been linked to tissue damage in rheumatoid arthritis sufferers. Tissue damage could result from the oxidant/ antioxidant balance's cyclical nature. Antioxidants should,

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therefore, be used in conjunction with other medications to treat rheumatic disease.[13,14] The present research work explains the development of an innovative transdermal cream of teriflunomide. Teriflunomide was already approved drug for the treatment of multiple sclerosis as oral tablets and is the primary metabolite of leflunomide, approved for the treatment RA as oral tablets. Literature substantiated that teriflunomide is responsible for the anti-RA effect of leflunomide. Hence, teriflunomide was selected directly as a drug molecule and transdermal cream of the same to treat RA.

MATERIALS AND METHODS

Materials and Reagents

Only pharmaceutical-grade materials were used during the development process. Teriflunomide drug (Batch No. TOM0030718; 99.96% w/w purity) was procured from MSN Laboratories Limited, India. Excipients were procured/ obtained as gift samples from reputed manufacturers and vendors. Labrafac Lipophile and Labrafac (oil phase vehicle), Emulcire, and Gelot (emulsifying agents and surfactants) were obtained as gift samples from Gattefosse. Dimethyl sulfoxide (permeation enhancer) was procured from PanReac, carbopol (gelling agent) was obtained as a gift sample from Lubrizol, Sodium hydroxide (pH adjuster and buffer), methyl and propyl parabens (preservatives), procured from Kredense. Analytical grade reagents and chemicals were used during development.

Methods

Physical characterization and identification

Teriflunomide API was characterized for organoleptic properties and also identified using the analytical methods described by the manufacturer. Teriflunomide drug powder was identified and confirmed using the HPLC method described by the manufacturer by comparing the RT (retention time) of the sample teriflunomide API with the RT (Retention time) mentioned in the sample COA.

Solubility studies

The solubility of API was studied in different solvents to understand the nature of the API, thereby choosing the appropriate excipients for the formulation development. The teriflunomide solubility in dimethyl sulfoxide, glycerin, propylene glycol, spans, tweens, water, etc., was determined semi-qualitatively. A specified fraction of teriflunomide was mixed with 10 mL of the solvent in 50 mL volumetric flask. The flasks consisting of the said mixture were sealed and agitated mechanically on a mechanical vibrator with and/or without heating, and observed the teriflunomide solubility.

Drug-excipient compatibility studies

Teriflunomide compatibility with the selected excipients was studied by performing the DSC of the drug, excipients, and the physical mixtures of the drug & excipient at a ratio that is intended to be used in the formulation. Three to Four milligrams of the mixture were placed in an aluminum pan and subjected to temperature up to 250°C with an increase of 10°C/min in a differential scanning calorimeter (Model: TA-501, Make: Shimadzu Corporation, Japan).

Formulation of teriflunomide transdermal cream

A very simple reproducible manufacturing process was developed for manufacturing the innovative teriflunomide transdermal cream. Oil phase of the cream formulation was manufactured by dissolving the teriflunomide in DMSO, labrafaclipophile and labrafac mixture between 70 to 80°C. Emulsire and Gelot were added and solubilized in the above oil mixture. Parabens were also solubilized in the same oil mixture. The aqueous phase is prepared by mixing the Carbopol dispersion (in water) and sodium hydroxide solution. The aqueous phase temperature was also increased to 70 to 80°C. When both phase's temperatures was between 70 to 80°C, the oil portion was transferred continuously to the aqueous portion under stirring. After mixing both portions, the cream temperature was decreased under continuous mechanical stirring. Eucalyptus oil and L-menthol were added under stirring when the temperature of the above cream was between 40 to 50°C. Finally, the pH of the cream was adjusted by adding the alkali solution.

Evaluation of the Formulations

Visual inspection

Visually the developed formulation was inspected for its color, clarity, gritty particles, homogeneity, and phase separation.

pH

Diluted cream (10%w/w) pH was recorded and reported. pH of the as-such cream was not recorded. One gram of the developed teriflunomide cream was diluted with purified water to 10 mL (10% w/w solution) and the pH of the same.

Viscosity measurement

Viscosity of the developed teriflunomide cream was studied using a rheometer with spindle CP-25 (Make: Antonpaar, Model: MCR 102E) at 25°C and shear rate: Ramp Linear 1 to 100 1/sec.

Spreadability

On a smooth-surfaced ceramic tile, 0.5 g of cream was placed inside a circle that had been previously marked with a 2.0 cm diameter to test the spreadability of the cream. A second known-weight ceramic tile was placed on the top of the first ceramic tile. Around 1000g weight was placed on the second ceramic tile for five minutes. Due to the weight of the second ceramic slab and 1kg weight, the cream spreads between the two slabs, and the increase in diameter of the cream was measured with electronic Vernier Callipers. The test was carried out three times and average reading was calculated, which is an indicator of the spreadability.

The results were presented as surface spreading area against the applied weight based on the mathematical equation below.

$$S_i = d^2 \mathbf{x} \frac{i}{4}$$

Where, S_i– Spreading area (mm^2), i – Applied weight (g) and d – Average diameter (mm)

Assay (Percentage purity)

The teriflunomide content in the formulation was determined in order to find out the %purity of teriflunomide in the formulation. 70 ppm solution of the teriflunomide cream was prepared by diluting the cream with buffer and acetonitrile mixture (30:70). The mixture was sonicated for 30 minutes with intermittent shaking every 5 minutes. The resultant solution was passed through the 0.45 µm nylon filter. The assay was performed using the validated HPLC method where Kromasil-100 C_{18} 250 nm x 4.6 nm, 5 µm column was used. While performing the analysis 1.0-mL/min was the flow rate, 35°C was the column temperature, 5 µL was the injection volume, and area readings were made at 250 nm to determine the teriflunomide percentage purity (Assay).

In-vitro drug release test (IVRT)

Vertical Franz diffusion (FD) cells having a 2.67cm^2 diffusion area were used to measure the teriflunomide release from the developed cream. A nylon net membrane with 0.20 µm and saline phosphate buffer (PBS), pH 7.4 was used as a semi-permeable membrane and receptor fluid, respectively. The receptor compartment was loaded with 20 mL of receptor medium and around 300 mg of test cream was spread on the surface of the nylon net membrane. The experiment was conducted with the magnetic bead rotating at 600 rpm, and the water bath's temperature was kept at 32 ± 0.5°C by the flowing water jacket. A 0.3 mL receptor fluid was drawn at every hour and analyzed. The experiment was carried out for 6 hours. The withdrawn receptor fluid was replaced with a similar volume of the fresh receptor medium (PBS) to maintain the specified volume and sink conditions. The withdrawn samples were analyzed using HPLC with Kromasil-100 C_{18} 250 × 4.6 nm, 5 µm column at 250 nm. Adequate measures were implemented to prevent the formation of air bubbles while withdrawing and replacing the receptor medium and the cumulative amount of the teriflunomide released was quantified against time for 6 hours.

In-vitro permeation test (IVPT)

A vertical Franz diffusion (FD) cell with a 2.67 cm^2 permeation area (internal diameter of the donor compartment) was used to study the *in-vitro* drug permeation from the developed innovative formulation. Strat-M, a synthetic membrane that mimics the human cadaver skin and saline PBS, pH 7.4 were used as a semi-permeable membrane and receptor fluid, respectively. About 20 mL of receptor medium and around 300 mg of test cream was placed in receptor and donor compartments, respectively. Test cream was spread on the surface of the Strat-M membrane.

The experiment was conducted with the magnetic bead rotating at 600 rpm, and the water bath's temperature at 32 ± 0.5°C by the flowing water jacket. A 0.3 mL receptor fluid was drawn at 0, 1, 2, 4, 6, 8,12, and 24 hours and analyzed. The withdrawn receptor fluid was replaced with an equal volume of fresh receptor medium to maintain the specified volume and sink conditions.

The withdrawn receptor fluid samples were analyzed using HPLC using Kromasil-100 C_{18} 250 x 4.6 nm, 5 µm at 250 nm. Adequate measures were taken to limit the formation of air bubbles while withdrawing and replacing the receptor medium. The total amount of teriflunomide permeated from the test cream formulation in 24 hours was calculated. A similar experiment was carried out with the Teriflunomide topical gel, 2% w/w to compare the drug permeability from the topical cream and gel.

Kinetics of the drug permeation

The teriflunomide permeation data (IVPT) was used to analyze the drug permeation kinetics and mechanisms. The data fitted in zero, first, Higuchi, Korsemeyer-Peppas, and Hixon Crowel mathematical models. By comparing the R2 values obtained from each of these mathematical models, the best-fitting model was chosen.

Zero–order equation

$$Q_t = k_0 t$$

Where,

Q_t - Percentage (%) of drug permeated

t - time (hr)

k_0 - permeation rate constant.

First–order equation

$$\textbf{Log } Q = \textbf{Log } Q_0 - \frac{Kt}{2.303}$$

Where,

Q_0 - initial concentration of drug,

K - first order rate constant,

T - release time;

Higuchi equation

$$Q_t = kH^{t1/2}$$

Where, kH represents the Higuchi release rate constant;

Korsmeyer-Peppas equation

Where,

Qt/Qe - Fraction of the drug released from the cream (donor compartment) into the receptor solution,

K - constant corresponding to the structural and geometric characteristics of the device and

N - the release exponent which indicates the mechanism of the drug release.

If, n= 0.5, n>0.5 and n=1, the mechanisms are is Quasi-Fickian diffusion mechanism, anomalous/ non-Fickian diffusion mechanism exists, and Zero order release one exists respectively.

Hixon crowel equation

The Hixon-Crowell model equation is as follows:

$$m_0^{1/3} - m_{left}^{1/3} = k_{H-C}t$$

Where,

m_{left} - the amount of drug left in the cream formulation,
t - time, and
k_{H-C} - Hixon-Crowell rate constant.

Stability study

Stability of the developed cream formulation was not studied as per the ICH guidelines and was not exposed to accelerated stability conditions. However, the samples were placed at RT for more than one year. After 1 year at RT, the developed cream was observed for its organoleptic properties, pH, etc.

Ex-vivo diffusion study Drug distribution across different layers of skin

Ex vivo permeation study of the cream was studied across the pork ear skin using the Franz diffusion apparatus. Each cell had a diffusion area of 2.9 sq.cm and pork ear skin was used as a semi-permeable membrane. Saline phosphate buffer was used as receptor fluid and experiment was carried out at 37 ± 0.5˚C.

Around 300 mg of cream was placed on the epidermal surface of the pork ear skin and the same was fixed between the donor and receptor compartments. At prescheduled time intervals, 0.3ml of the receptor solution was withdrawn from the receptor compartment and replaced with a freshly prepared receptor medium. Drug concentration at each time point was quantified and the graph between the cumulative amount of drug permeated from a unit area of the skin in unit time versus time (h). The average flux (J_{ss}, µg/cm^2.h), %CDR and cumulative amount of drug release (µg) was estimated.

After completion of the permeation study, the semi-permeable membrane, i.e., skin samples, were collected carefully. Gel present on the epidermal surface of the skin was collected and transferred volumetric flask consisting of the fresh receptor fluid. The skin surface are also washed with fresh receptor fluid to collect excess drugs from the surface. The same was sonicated and the quantity of the drug was held on the skin surfaces using well well-developed HPLC analytical method.

After collecting the cream from skin surfaces, the skin samples are chopped as small pieces and transferred into the receptor fluid. The same was homogenized for 30 minutes. Then it was centrifuged at 8000 rpm for 20 minutes. Methanol was used to extract the drug held in the skin. The amount of teriflunomide held in the skin was measured using well developed HPLC analytical method.

In-vivo acute anti-inflammatory activity

The acute anti-inflammatory efficacy of the developed formulation was studied in male Wistar rats (190–230g). The rats were handled as per the procedures framed by the ethical committee, Department of Pharmacology, Vishnu Institute of Pharmaceutical Science and Research, Telangana. Selected rats were randomly distributed as four groups consisting of six rats in each group, namely placebo control (G1), disease control (G2), and two test groups [topical route (G3) and oral route (G4)].

Oedema was induced in G2-G4 groups by administering carrageenan solution, 5% w/v, (0.02 mL) to the left hind paw of the rat's sub-plantar region. In G1, a similar volume of saline was injected as described in G2-G4 groups. The test formulations were applied 60 min before injecting the carrageenan. The paw volumes were measured at 0 and after 240 minutes (after 4 hours) using a Plethysmometer and mercury displacement agent.

Statistically compared the variation in the paw volumes of the rats present in the test and control groups. Both ANOVA and student-t tests were applied to find out the difference was significant or not.

RESULTS AND DISCUSSION

Characterization of the API

Physical appearance and identification

Teriflunomide appeared as a white to slightly yellow color powder and was identified by comparing the RT (retention time) of the teriflunomide against the API standard using HPLC assay method.

Solubility studies

The solubility data of teriflunomide is presented in Table 1. Teriflunomide exhibited higher solubility in Dimethyl sulfoxide followed by other oily solvents like Labrafac PG, Maisine CC, etc. It has exhibited good solubility in surfactants too. Considering the solubility and other characteristics of the solvents, oil vehicles, and drug product requirements, DMSO, Labrafac PG, and Labrafaclipophile were selected for the development of the teriflunomide topical dosage form.

Drug excipient compatibility studies

The DSC thermograms of the mixtures of API and individual excipients did not exhibit new peaks in the mixture and the absence of the peak. However, few API and excipients mixtures show variation in the melting point of API/ excipients. No interaction was observed in the formulation when it was at RT for more than a year. This indicates that the drug does not have any interaction with the excipients used in the development/ manufacturing of the topical cream. The thermograms of API and excipients are shown in Figure 1.

Characterization of the Developed Formulation

Physical appearance

Formulation visually appeared as white to off-white viscous homogenous cream without any gritty particles and observed that there is no phase separation on storage at room temperature, as shown in Table 2. PLM image of the teriflunomide topical cream is given in Figure 2.

pH

pH of the cream diluted with water to 10% was measured and found within the range of 6.0 ± 0.5.

Table 1: Solubility data of the teriflunomide indifferent organic and aqueous solvents

S. N.	*Name of the solvent*	*Manufacturer*	*Role*	*Solubility (mg)*	*Observation*
1	Sunflower oil	AKK Kamani	Oil vehicle	2.5 mg/g	Insoluble
	Sunflower oil heated at 80◦C			5 mg/g	Soluble
2	Hydrogenated vegetable oil	AKK Kamani	Oil vehicle	2.5 mg/g	Insoluble
	Hydrogenated vegetable oil heated at 80◦C			5 mg/g	Soluble
3	Labrifil M 1944 CS	Gatteffosse	Non-ionic surfactant	2.5 mg/g	Insoluble
	Labrifil M 1944 CS heated at 70◦C			1.5 mg/g	Soluble
4	Capryol 90	Gatteffosse	Non-ionic surfactant	2.5 mg/g	Soluble
	Capryol 90 heated at 70◦C			10 mg/g	Soluble
5	Transcutol P	Gatteffosse	Solvent - solubilizer & permeation enhancer	10 mg/g	Soluble
	Transcutol P heated at 70◦C			27.5 mg/g	Soluble
6	Emulcire 61 WL 2659 (Solid flaks) heated at 70◦C	Gatteffosse	O/W emulsifier	22.5 mg/g	Soluble
7	Labrifil M 2130 CS (GREEZY) heated at 70◦C	Gatteffosse	Non-ionic water-dispersible surfactant	2.5 mg/g	Soluble
8	Gelot 64 (Solid flaks) heated at 70◦C	Gatteffosse	Non-ionic oil-in-water emulsifier	15 mg/g	Soluble
9	Dimethyl sulfoxide	Panreac	Solvent	40 mg/mL	Soluble
10	Isopropyl alcohol	Avantor	Solvent	2.9 mg/mL	Insoluble
11	Propylene glycol	Altapure	Solvent	6.6 mg/mL	Insoluble
12	Poly oxyl 35Castor Oil (Kolliphore EL)	BASF	Non-ionic surfactant	3 mg/mL	Soluble
13	Tween 80	Emprov EXP	Non-ionic surfactant	5 mg/mL	Soluble
14	Kolli solve (MCT)	BASF	Emollient	4 mg/mL	Insoluble
15	Kolli solve (GTA)	BASF	Solvent-Solubilizer	4 mg/mL	Insoluble
16	Kolli cream (OA)	BASF	Emollient oil, Solubilizer and permeation enhancer	4 mg/mL	Insoluble
17	Kolli cream (3C)	BASF	Medium polar oil for topical	2.5 mg/mL	Insoluble
18	Kolli cream (IPM)	BASF	solvent - skin penetration enhancer solvent	2.5 mg/mL	Insoluble
19	Kolli cream (DO)	BASF	Solubilizer & penetration enhancer	2.5 mg/mL	Insoluble
20	Poly oxyl 35Castor Oil Heated at 60-100°C	BASF	Non-ionic surfactant	16 mg/mL	Soluble
21	Coconut oil Heated at 60-100°C	Parachut	Oil vehicle	7.5 mg/mL	Soluble
22	1-methyl 2-pyrrolidone	Merk	Solvent-Solubilizer	125 mg/mL	Soluble
23	Labrafac PG (70-90◦C)	Gatteffosse	Oil vehicle	30 mg/g	Soluble
24	LabrafacLiphophile WL 1349 (70-90◦C)	Gatteffosse	Oil vehicle	25 mg/g	Soluble
25	Maisine CC (70-90◦C)	Gatteffosse	Oil vehicle	37.5 mg/g	Soluble
26	Peceol (70-90◦C)	Gatteffosse	Oil vehicle	22.5 mg/g	Soluble
27	Labrafil-M-2125 (70-90◦C)	Gatteffosse	Non-ionic water-dispersible surfactant	20 mg/g	Soluble
28	Labrafil-M-1944 CS (70-90◦C)	Gatteffosse	Non-ionic water-dispersible surfactant	15 mg/g	Soluble
29	Labrasol (70-90◦C)	Gatteffosse	Non-ionic oil-in-water surfactant	32.5 mg/g	Soluble

Viscosity

Rheometer was used to measure the viscosity of the teriflunomide cream and was found as 6141.5 mPa.s at 25°C. The torque values were found in the range of 15 to 95% of the base scale.

Spreadability

The success rate of the semisolid formulations depends on the way the patient spreads the formulation on the affected area. The extent of standard dose delivery of the drug depends on the extent of evenly spread the drug on the skin surface. A

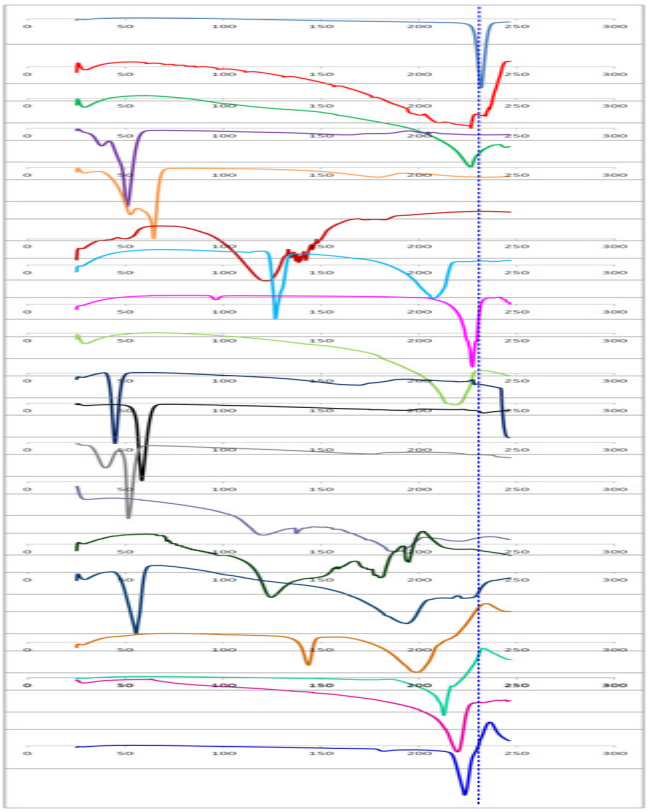

Figure 1: DSC thermo grams of API and physical mixture of API and excipients (From top to bottom): 1. Teriflunomide API, 2.Teriflunomide:Labrafac-pg, 3. Teriflunomide:Labrafaclipophile, 4. Teriflunomide+ Emulcire (2:9), 5. Teriflunomide+ Gelot 64 (2:3.6), 6. Teriflunomide+ DMSO (2:10), 7. Teriflunomide+ Methyl Paraben (2:0.5), 8. Teriflunomide+Propyl paraben(2:0.06), 9. Teriflunomide+ Coconut Oil(2:3), 10. Teriflunomide+ L-Menthol (2:5), 11. Teriflunomide+Stearic acid (2:9) 12. Teriflunomide+Cetosteryl alcohol (2:6), 13. Teriflunomide+Tween80 (2:4), 14. Teriflunomide+Glycerin (2:10), 15. Teriflunomide+Span 60 (1:1), 16. Teriflunomide+Cholesterol (1:0.5), 17. Teriflunomide+ Soyalecithin (1:0.199), 18. Teriflunomide+ Eucalyptus oil (2:3), 19. Teriflunomide+Carbapol 10NF (2:0.5)

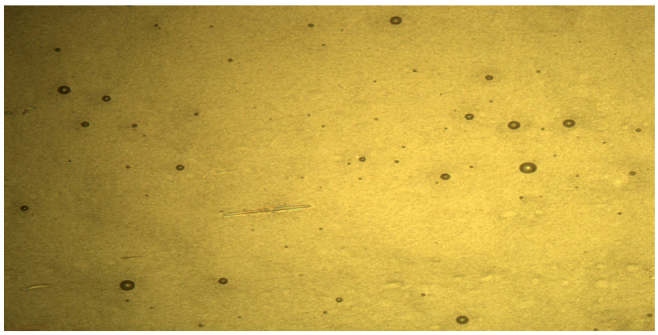

Figure 2: PLM image of the teriflunomide topical cream, 2%w/w

formulation with the ideal consistency helps in administering the right dose and/or delivering the intended location. The spreadability of the developed formulation was identified as 67.5 mm/0.5 g.

Table 2: Comparison of the physical properties of the initial and 12 months old samples of the developed cream formulation

Stability conditions		*RT*	*25°C/ 60% RH*
Stage		*Initial*	*After 12 months*
S No	*Parameter*	*Observations*	
1	Description	White to off white viscous cream	White to off white viscous cream
2	pH	6.28	6.22
3	Phase separation	Not observed	Not observed
4	Gritty particles	Not observed	Not observed

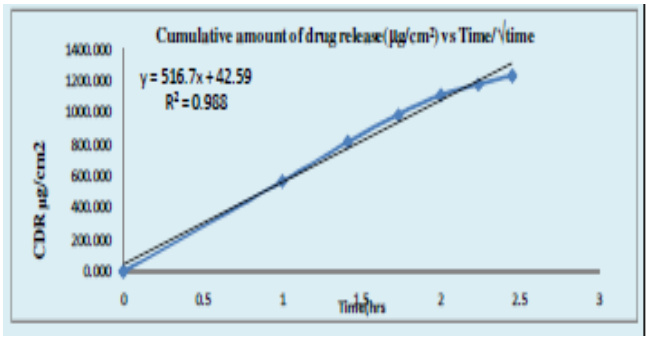

Figure 3: *In-vitro* drug release plot of the teriflunomide topical cream, 2% w/w

Assay (Percentage purity)

The assay (% purity) of the developed cream was quantified as triplicate with the validated HPLC method and was found within the range of 90.0 ± 10.0% w/w. The assay of the cream formulation was found within the specified range.

In-vitro drug release test (IVRT)

IVRT study demonstrated the teriflunomide release across the synthetic semi-permeable membrane, i.e., nylon net, was satisfactory. The release data was constructed as a plot between the cumulative amount of drug released (µg/cm^2) and the square root of time (Figure 3).

In-vitro permeation test (IVPT)

A plot was constructed between the cumulative amount of the teriflunomide permeated from a unit of the surface area (µg/cm^2) into the receptor fluid and time (hr). The flux (J_{ss}, µg/cm^2/h) of the developed cream formulation was considered from the slope (steady state portion) of the linear portion of the above plot. The average flux (J_{ss}, g/cm^2/h), average percentage of drug permeated (%) and the average cumulative amount of drug permeated (g/cm^2) are reported in Table 3. The obtained results substantiated that the cream formulation was effectively permeating across the skin.

Kinetics of the drug permeation

Permeation study data (IVPT) of the developed formulation was used to find out the permeation kinetics and mechanisms of the teriflunomide from the developed formulation. The data was fitted in different kinetic models, i.e., zero, first, Higuchi, Hixon Crowel, and Korsemeyer –Peppas models.

Table 3: *In-vivo* permeation data of the teriflunomide topical cream, 2%w/w

S No	*In-vivo permeation parameter*	*Observation*
1	Avg flux (Jss, µg/cm2/h)	68.49
2	Avg percentage drug release (%)	74.52
3	Avg cumulative amount of drug permeated (g/cm^2)	1553.97

Drug transport from pharmaceutical systems is governed by a number of physical and chemical laws, which makes it challenging to assign an appropriate mathematical model to the events that take place. If the drug is released slowly from a pharmaceutical dosage form that does not dissolve, then zero-order kinetics may be used. The first-order model will be adopted when the drug is poorly soluble and embedded in the water-soluble matrix and the drug release follows the dissolution instead of diffusion. The Higuchi equation is based on a number of presumptions, including that the drug's initial concentration in the formulation is more than its solubility, that it transfers only in one dimension, that system swelling and drug dissolution are negligible, that drug diffusivity is constant, and that sink conditions are met. When the drug is delivered on parallel planes of the drug form surface, such as in the case of a tablet, when the size decreases proportionately and the geometric shape remains constant, the Hixon-Crowell model is used.[15,16]

When more than one kind of drug release mechanism is involved, or the drug release phenomena is unknown, Korsemeyer-Peppas mathematics is helpful in describing drug release from a polymeric system by taking non-Fickian mechanisms into account.[17-19]

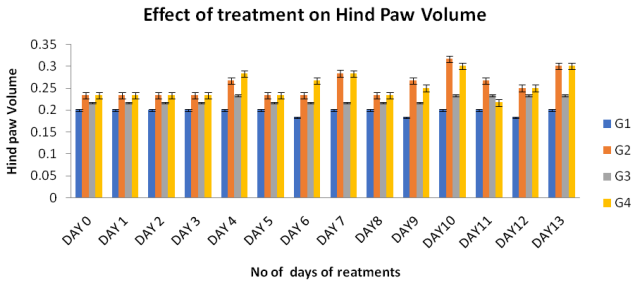

Figure 4: *In-vivo* anti-inflammatory effect of the teriflunomide topical cream, 2% w/w- Hind paw volume measurement

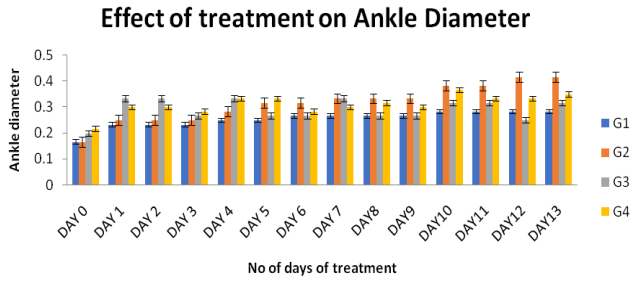

Figure 5: *In-vivo* anti-inflammatory effect of the teriflunomide topical cream, 2% w/w- Ankle diameter measurement

Table 4: Mathematical models and respective regression coefficient values of the teriflunomide topical cream, 2% w/w

S. No.	*Mathematical model*	*R^2*	*N*
1	Zero order	0.9952	N/A
2	First order	0.9733	N/A
3	Higuchi	0.9524	N/A
4	Hixon-crowell	0.8140	N/A
5	Korsemeyer- peppas	0.9028	2.691

The best-fitting model was identified by comparing the R^2 values of these mathematical models. The R^2 values depict the kinetic model, which is appropriate for the drug permeation. The R^2 values are presented in Table 4.

The R^2 values substantiated that the drug release from the topical cream was best fitting in the zero order and Higuchi equations which were in line with the kinetics of the many reported topical creams. The model, which has an R^2 value close to 1 will be considered as the release mechanism of the polymeric system. For the present cream formulation, the best kinetic model was identified as zero-order followed by the Higuchi model, as the plots exhibited high linearity, confirming mainly the diffusion process. The results obtained in the Korsmeyer-Peppas model for the developed cream formulation also confirmed the same.

According to this model, the drug release is Fickian diffusion when the n value was ≥ 0.5. If the n value lies between 0.5 to 1.0 indicates anomalous passing. In the present cream formulation, the n value was above 0.5, i.e., n=2.691confirmed that the teriflunomide permeation from the developed cream formulation was controlled by several processes, not by an individual process.

Stability studies

The samples do not show any change in physical parameters, such as organoleptic properties, pH, etc, when the samples are stored at RT for more than a year. This was confirmed when compared with initial values. This substantiated that the sample is stable at RT for a year. However, formal stability studies as per ICH guidelines are to be taken up as we progress.[20,21]

In-vivo acute anti-inflammatory activity[22,23]

The acute anti-inflammatory activity of cream formulation and oral teriflunomide suspension was investigated by the well-established carrageenan-induced rat paw edema method, and the results obtained are shown in Figures 4 & 5. Edema inhibition in carrageenan-induced rat paws was studied by measuring the ankle volume and ankle thickness. The results substantiated that the significant percent inhibition of the edema is more with the topical formulation rather than the oral suspension.

CONCLUSION

The study results substantiated that the development of the innovative topical dosage form of teriflunomide was positive and promising. The study established the stability of the developed formulation and other parameters met the required

standards. *In-vivo* efficacy study of the developed formulation confirmed that the formulation is effective against acute inflammation. The authors are confident that the developed topical formulation of teriflunomide addresses the adverse effects associated with oral leflunomide and teriflunomide and also addresses the early morning joint stiffness associated in RA patients. However, the efficacy of this developed topical formulation against morning joint associated in RA patients and advantages over the oral tablets of the leflunomide/ teriflunomide are to be established by performing the preclinical and clinical studies as required.

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