Design And Development of Emulsomes with Improved Skin Permeation for The Topical Treatment of Rheumatoid Arthritis

Mohammad Sameer Ansari[1], Dr. Vaseeha Banu T. S[2]
[1]*Assistant Professor, MMU College Of Pharmacy, Ramanagaram-562159*
[2]*Principal/Professor, MMU College Of Pharmacy, Ramanagaram-562159*

***Abstract*—Arthritis is main cause of disability in humankind in developed and developing countries. Among which rheumatoid arthritis and gouty arthritis are most prevalent ones. Immunosuppressants (Tacrolimus) and xanthine oxidase inhibitors (Febuxostat) are the choice of treatment for RA and gouty arthritis respectively. However, Tacrolimus and Febuxostat have several limitations of gastrointestinal related disturbances and low oral bioavailability due to first pass metabolism and low aqueous solubility. Thus, to improve bioavailability, transdermal drug delivery systems of Tacrolimus and Febuxostat were developed as their treatment options. Leflunomide, an isoxazole derivative and inhibitor of de novo pyrimidine synthesis, represents a new class of DMARDs. Leflunomide is an immunosuppressant medication used in the treatment of active rheumatoid arthritis (RA). Leflunomide prevents the expansion of activated autoimmune lymphocytes by inhibiting dihydroorotate dehydrogenase, an enzyme essential for fresh (de novo) synthesis of pyrimidine.**

Emulsome is an advance nanocarrier technology for poorly aqueous soluble drugs. It possesses both emulsion and liposomes feature Emulsomes offers an effective topical drug delivery system owing to its potential of high retention flux as well as high skin retention of drug and hence enhanced activity and reduce skin irritation.

It involves the application of drug to the skin to obtain the desired therapeuticeffect for the treatment of disease far from the site of application. Topical drug delivery renders accurate amount of drug from the skin to achieve systemic action. Its non- invasive nature is additional advantages which provide continuous drug delivery to skin similar to intravenous administration. The purpose of this study was to formulate and evaluate Leflunomide Emulsomes for the treatment of Rheumatoid Arthritis. Leflunomide, an isoxazole derivative and inhibitor of de novo pyrimidine synthesis, represents a new class of DMARDs. Leflunomide is an immunosuppressant medication used in the treatment of active rheumatoid arthritis (RA). RA is an autoimmune disease in which the body's own immune system mistakenly attacks the joints, causing inflammation and damage to the joints. Leflunomide is a disease modifying antirheumatic drug (DMARD) that modulates the immune system to reduce inflammation and slow down disease progression. Leflunomide is a prodrug that is converted to its active metabolite A77 1726 (referred to as M1) in the body. The short half-life and high frequency of administration of Leflunomide makes it a suitable candidate for designing Topical drug delivery system. Topical Emulsomes prolong residence time of drugs, improve bioavailability, and facilitate drug delivery through systemic route. With this objective, Topical Emulsomes of Leflunomide as a model drug was prepared for the treatment of Rheumatoid Arthritis.

***Index Terms*—Emulsomes, Lecithin, Cholesterol, Rheumatoid Arthritis**

I. INTRODUCTION:

1.1 Rheumatoid Arthritis

Rheumatoid joint inflammation (RA) is a persistent, even, provocative immune system sickness that starts in little joints & advances to skin, eyes, heart, kidneys & lungs. Joint bone & ligament are oftentimes annihilated, ligaments & tendons debilitate. All of this joint harm brings about deformations & bone disintegration, which are normally intensely excruciating for patient. Morning firmness of impacted joints for over 30 minutes, weakness, fever, weight reduction, delicate, enlarged, and warm joints, rheumatoid knobs under the skin are normal side effects of RA. [1]

Disease typically shows up b/w the ages of 35 and 60, with times of abatement & fuel. It can like-wise

influence small kids before the age of 16, which is known as adolescent RA (JRA), which is like RA with the exception of that no rheumatoid component is available the pervasiveness of RA in the West is assessed to be 1-2% & 1% around the world.[2]
Clinically, RA varies from osteo-arthritis (OA) in that impacted regions in RA are proximal inter-phalangeal (PIP) & meta-carpo-phalangeal (MP) joints, while OA commonly influences distal inter-phalangeal (Plunge) joint. The most well-known kind of joint pain is osteoarthritis (OA), which is brought about by mileage instead of an immune system condition. It isn't hurtful to the lungs, heart, & invulnerable framework. Moreover, not at all like RA, which is even, OA regularly influences just a single side of body. Another recognizing highlight is that RA patients experience persevering morning firmness for no less than 60 minutes. Morning firmness is normal in OA patients; however, it typically resolves or diminishes inside 20-30 minutes.[3]

Epidemiology of RA

Rheumatoid arthritis (RA) is a widespread autoimmune disease with a global impact on public health, affecting individuals across diverse demographic and geographic settings. Globally, the incidence of RA varies, although it is often greater in industrialised nations. This difference in frequency can be attributed to a variety of variables, including genetics, insufficient reporting in some regions of the world, and susceptibility to environmental risk factors. RA exhibits variability in prevalence across different regions and populations. Globally, it is estimated that approximately 0.5-1% of the adult population is affected by RA, making it one of the most common autoimmune inflammatory arthritis conditions.[4]Both modifiable lifestyle-related variables and unmodifiable characteristics, such genetics and sex, are common risk factors for RA. The proportion of female to male RA cases is 3:1, meaning that women are more likely than males to have the disease.

Furthermore, smoking, being overweight, and being among pollutants are risk factors. The etiology of RA is multifactorial, involving a complex interplay of genetic, environmental, and hormonal factors. Several identified risk factors contribute to the development of RA like genetic predisposition, environmental triggers, hormonal factors and age etc. Genetic predisposition means the individuals with a family history of RA are at an increased risk of developing the condition.[5]

Pathophysiology

Osteoarthritis is described by an ever-evolving degenerative outpouring of ligament misfortune that prompts bone harm. Sub-chondral growths, osteophytes, & sub-chondral plate thickening are normal discoveries. Interleukin-6, monokines, interferon-initiated protein-10, & macrophage chemo-tactic protein all actuate proteo-lytic chemicals that corrupt joint collection, including network metalloproteinases, serine proteases, & cysteine proteinases Calcification of encompassing articular ligament decreases thickness of the cartilaginous network & in the long run annihilates it. Age is likewise connected with a decrease in chondrocyte capability, which expands weak-ness to osteo-arthritic degeneration.[6]

Rheumatoid joint pain side effects are normally more extreme than osteoarthritis side effects. Rheumatoid joint pain is a fundamental & persistent provocative condition made by an immune system reaction a trigger in climate. Endothelial cell initiation & synovial cell hyperplasia go before ligament and, at last, bone debasement.
Pathology creates because of unusual fiery arbiter creation, (for example, growth corruption alpha, interleukins 1, 6 & 8 & others following openness to an antigenic microbe).[7]

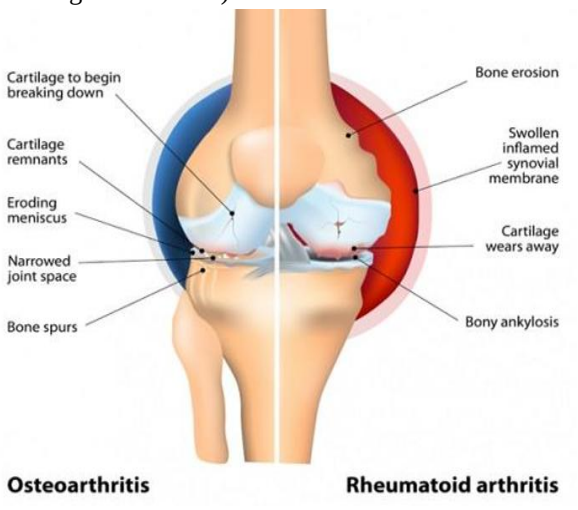

Fig 1: Differences between Osteoarthritis and Rheumatoid Arthritis

Inflammation pathway to rheumatoid arthritis[8]

Rheumatoid arthritis is thus characterized by symmetric polyarticular inflammation of the synovium, typically of the small joints of the hands (MCP and PIP), wrists and feet. This inflammation results in pain and stiffness, and can lead progressively to joint damage causing deformities and loss of function. Associated organ damage also contributes to severe disability. Chronic inflammation secondary to RA, additionally, may result in an increased risk of cardiovascular disease and changes in bone metabolism. RA if left untreated may progress to joint destruction, subluxation and severe disability.

Signs and Symptoms[9]

Below mentioned conditions comprise of the symptoms of RA

- Joint pain
- Rigidity in joints
- Soreness or inflammation in the joints
- Symmetry in symptoms such as both knees or hands
- Reduction in body weight
- Pyrexia
- Weariness and weakness

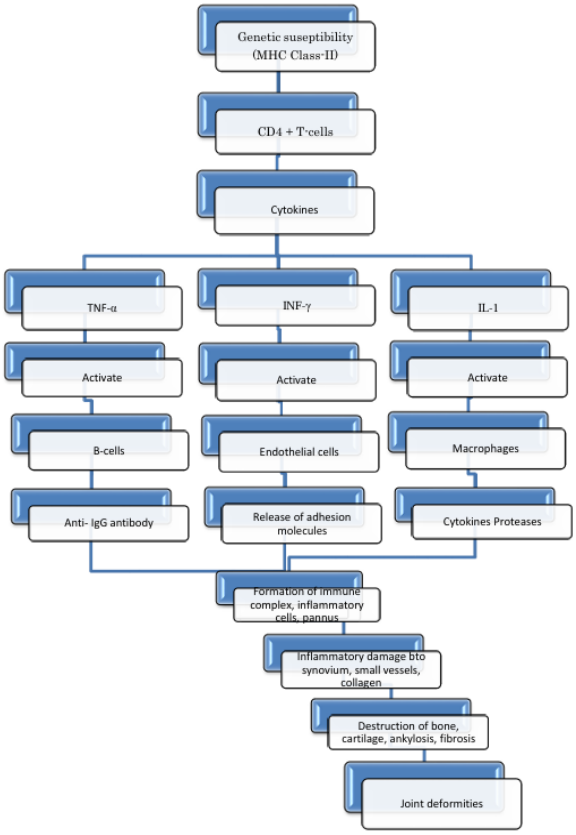

Fig 2: Inflammation pathway to rheumatoid arthritis

1.2. Classification of Rheumatoid Arthritis

Table 1: The 1987 Revised Criteria for Classification of Rheumatoid Arthritis

CRITERIA	DEFORMITY
a. Morning stiffness	1 hour of stiffness in and around the joints before maximum improvement
b. Arthritis affecting three or more joints	At least three joint areas are simultaneously inspected by a physician: proximal, interphalangeal, metacarpophalangeal, wrist, elbow, knee, ankle, and metatarsophalangeal joints (right or left).
c. Arthritis that affects the joints of the hands.	Wrist, metacarpophalangeal, or proximal interphalangeal joint arthritis.
d. Symmetric arthritis	On both sides of the body, the same joint regions are involved at the same time.

e. Rheumatoid nodules	A clinician may see subcutaneous nodules on bony prominences, extensor surfaces, or juxta-articular regions.
f. Serum Rheumatoid Factor	Any procedure that has resulted in a positive result in less than 5% of normal control subjects demonstrating abnormal quantities of serum rheumatoid factor.
g. Radiographic changes	Erosions or unambiguous bone decalcification localized in or most marked near to the affected joints are typical RA alterations on postero-anterior hand and wrist radiographs.

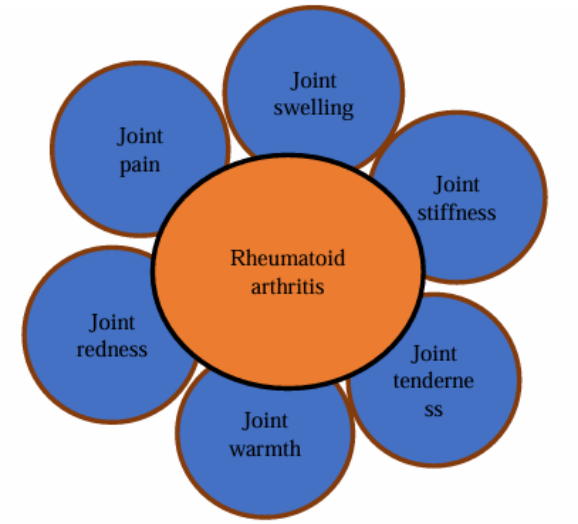

Fig 3: Symptoms appear in rheumatoid arthritis

1.3. Rheumatoid arthritis: Diagnosis and management [10]

The following are some standard procedures for diagnosing rheumatoid arthritis:

➢ Physical examination and medical history:

Your symptoms, their duration, and any family history of arthritis will be inquired about by the doctor. A comprehensive physical examination will be performed to look for warmth, soreness, and swelling in the joints.

➢ Blood Tests: Rheumatoid Factor (RF):

A positive result on an RF test is detected in certain RA patients, but it is not conclusive as it can also be found in some healthy individuals and people with other conditions. Antibodies against the cyclic citrullinated peptide, or anti-CCP antibodies, are more specifically directed against RA. Increased body inflammation is indicated by elevated levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)

➢ Imaging Studies: X-rays:

These can show alterations in the condition of joints over time. Magnetic Resonance Imaging (MRI): This modality can assist in determining the extent of joint inflammation and offers fine-grained images of soft tissues.

➢ Analysis of Synovial Fluid:

Extracting synovial fluid from a symptomatic joint can assist in identifying whether inflammation is brought on by RA or another factor.

➢ Clinical Criteria:

Based on the quantity and duration of symptoms, the American College of Rheumatology (ACR) has developed criteria for classifying RA.

➢ Joint Ultrasound:

This imaging modality allows for the real-time visualization of joint damage and inflammation.

1.4. DRUG DELIVERY THROUGH SKIN

Along with oral route, the transdermal route now ranks amongst the most successful innovative research areas in drug delivery. A three-day scopolamine patch to

treat motion sickness that delivers scopolamine systemically was the first transdermal system approved for use in the United States in 1979. Nicotine patches later became the first transdermal blockbuster. Today, there are over 25 transdermal delivery systems for such drugs as estradiol, fentanyl, lidocaine and testosterone, clonidine, rivastigmine; combination patches containing more than one drug for contraception and hormone replacement; and iontophoretic and ultrasonic delivery systems for analgesia.[11]

Advantages of transdermal drug delivery [12]

- Metabolic activity in the skin is limited compared to that in the liver. Therefore, transdermal drug delivery can be used when there is a significant first-pass effect of the liver that can prematurely metabolize drugs given by oral route.
- It is a non-invasive drug delivery mode and therefore has advantages over hypodermic injections, which are painful, generate dangerous medical waste and pose the risk of disease transmission by needle re-use, especially in developing countries
- A continuous delivery profile can be achieved. Peak and valley levels in the serum observed in oral delivery are avoided.
- Being non-invasive they can be self-administered. They can provide release for long periods of time (up to one week).
- The route of drug delivery being the skin a relatively large surface area for absorption is available.
- They also possess improved patient compliance as they are non-invasive and convenient to handle.

Challenges for transdermal drug delivery [14,15]

- Since the natural function of the skin is to protect the body from unwanted effects from the environment, the most important limitation in transdermal application of drugs is the skin barrier. The skin is a major barrier for permeability refractive to nearly all except small lipophilic molecules
- It is difficult to achieve high and constant drug flux through the skin.

Skin as a barrier to drug delivery

Anatomically, the skin is the largest organ of the body, continuous, with a total area of about 20 square feet. The skin functions to protect us from microbes and the elements, helps regulate body temperature and permits the sensations of touch, heat, and cold. The skin is composed of following anatomically distinct layers.

i. Epidermis: It is the outermost layer of skin. It provides a waterproof barrier. This layer is made up of

a. stratum corneum

b. viable epidermis

ii Dermis: It lies beneath the epidermis. It contains tough connective tissue, hair follicles, and sweat glands.

iii. Subcutaneous or the fat layer

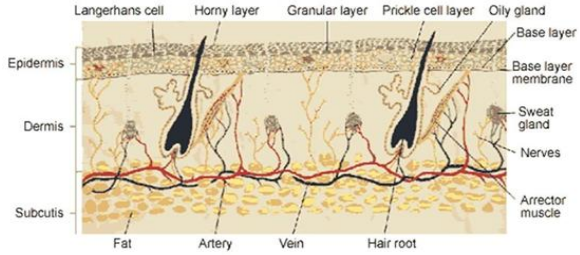

Fig 4: Layers of the skin

1.. Epidermis: it is the uppermost multi-layer of the skin, composed of stratified keratinized squamous epithelium .it contains four principal types of cells, such as keratinocytes (90%), melanocytes, Langerhans cells and Merkel cells. The thickness of epidermis varies depending on the cell size and the number of cell layers ranging from about 0.8 mm on the palms and soles down to 0.6 mm on the eyelids. The epidermis is divided into 5 sub -layers namely.

a) Stratum corneum (horny layer

b) Stratum lucidum

c) Stratum granulosum (granular layer)

d) Stranum spinosum (prickly cell layer)

e) Stratum germinativum (basal layer and dermo epidermal junction)

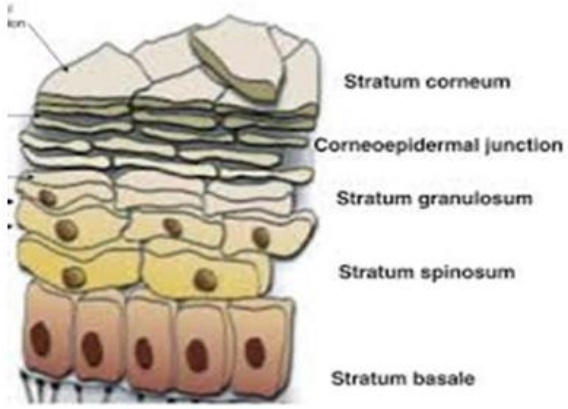

Fig 5: Layers of epidermis

2. Dermis: Dermis is the second deeper region lying in between the epidermis and subcutaneous fatty region. it is formed from connective tissues containing collagen and elastin fiber including few cells as fibroblasts, macrophages and adipocytes. blood vessels.

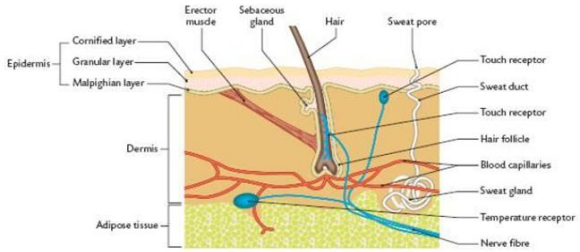

Fig 6: Components of dermis

3.hypodermis: it is a subcutaneous layer which lies deep to the dermis, but not the part of skin, this layer consists of areolar and adipose tissue known as special fascia attaching the dermis to the underlying structures.

4. skin appendages: these are also known as skin derivatives which include hair follicles, associated sebaceous glands

II. RESULT AND DISCUSSION:

Pre-formulation studies:

Determination of absorption maximum (λ_{max})

The λ_{max} of the Leflunomide was found to be 261 nm in pH 6.8 phosphate buffer. The graph of absorption maximum (λ_{max}) is showed in figure no 7

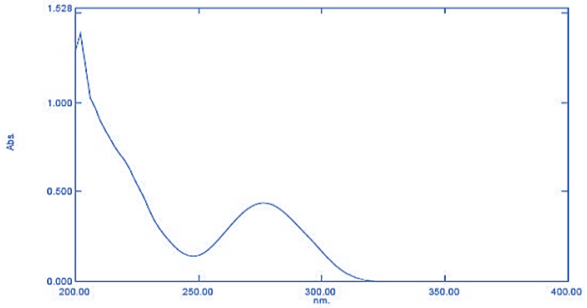

Figure 7: UV spectrum of Leflunomide in pH 6.8 phosphate buffer

Calibration curve of Leflunomide:

The absorbance was measured in a UV spectrophotometer at 208nm in pH 6.8 phosphate buffer. The linear plot between concentrations versus absorbance showed that Beer-Lambert's law was obeyed in concentration range of 50-250 µg/ml in pH 6.8 phosphate buffer (figure 25). The methods have shown good reproducibility. Correlation coefficient (r^2) values were found to be 0.9993 in pH 6.8 phosphate buffer, which indicate linearity. The data of calibration curve of Leflunomide is reported in table 5.

Table 2: Spectrophotometric data of Leflunomide in pH 6.8 phosphate buffer

Sl. No	Concentration (µg/ml)	Absorbance at 261nm			Average	Standard deviation (±SD)
		Trial1	Trial2	Trial3		
1	0	0.000	0.000	0.000	0.000	0.0000
2	50	0.147	0.151	0.148	0.148	0.0017
3	100	0.308	0.308	0.307	0.308	0.0019
4	150	0.489	0.488	0.487	0.488	0.0022
5	200	0.642	0.643	0.643	0.643	0.0029
6	250	0.821	0.823	0.828	0.824	0.0032

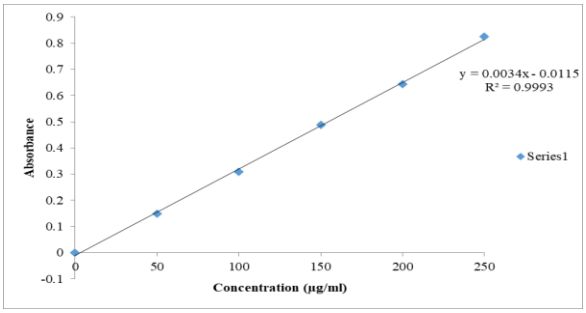

Figure 7: Calibration curve of Leflunomide in pH 6.8 phosphate buffer

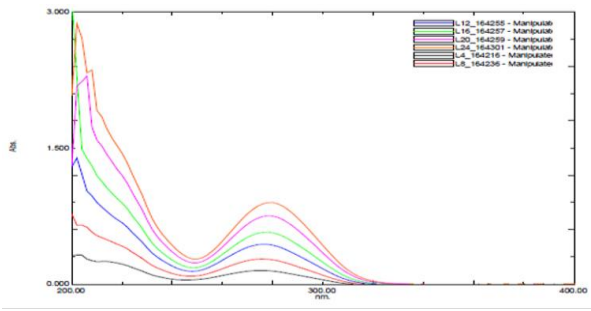

Fig 8: Linearity Curve of Standard curve of Leflunomide

Table 2: Optical characteristic and statistical data of the proposed method

Parameters	Values
	pH 6.8 phosphate buffer
λmax (nm)	261nm
Beer's law limit (µg /ml)	50-250
Slope (b)	0.0374
Intercept (a)	0.0094
Regression equation; (y= a+bx)	y=0.0374x-0.0094
Correlation coefficient (r^2)	0.9993

Melting Point Determination:
Melting point of pure drug Leflunomide was found to be 166.5°C and it is within the range specified in the official limits (165-168 °C), which complied with official standards, indicating purity of the drug sample.

Drug-excipient compatibility studies:
Studies of drug-excipient compatibility represent an important phase in the pre formulation stage of the development of all dosage forms. The potential physical and chemical interactions between drugs and excipients can affect the chemical, physical, therapeutical properties and stability of the dosage form. Fourier transformed infrared (FTIR) spectroscopy was selected for checking of drug-excipient compatibility

Fourier transformed infrared (FTIR) spectroscopy:
Because the frequency of vibrations within a chemical structure is very sensitive to how the atoms are arranged and how the atoms interact with neigh boring functional groups, not only can FTIR provide a detailed fingerprint of pharmaceutical substances of different physical forms, but also it can detect changes in hydrogen-bonding pattern of amorphous materials.
Infra-red spectrum of drug Leflunomide and polymers were recorded over KBr disc method and obtained spectra were shown in the figure. In pure drug Leflunomide, the characteristic absorption band at 3365.43$cm^{-1}$was due to the stretching vibration of N-H group. The absorption band at 3074.93 was due to the Ar-CH group, C-C symmetric band at 1707.78cm^{-1}, and (C-N) stretching vibration at810.37cm^{-1}. This further confirms the purity of Leflunomide. All the characteristic peaks of Leflunomide were present in the spectrum of drug and excipients mixture, indicating compatibility between drug and polymer. Hence, IR spectrum confirmed that there was no significant change in the chemical integrity of the drug.

III. CONCLUSION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that primarily affects the joints, leading to a spectrum of clinical manifestations. The disease's course is characterized by joint inflammation, pain, and stiffness, with potential involvement of multiple organ systems.
Emulsomes have structural similarity with the chylomicrons (natural lipoprotein of the body) and consequently expected to mimic these lipoproteins in behavior. These diminutive lipids like particles are frequently taken through endogenous lipid absorption mechanism through enterocytes of GIT tract Intestinal absorption of long-chain triglyceride from enterocytes is a complex incident that includes the co-ordination of synthesis of apolipoproteins and lipids and their intracellular assembly into mature lipid-containing particles.
Leflunomide is a disease-modifying antirheumatic drug (DMARD). It works by stabilizing the over-active cells in the immune system that cause inflammation in the joints. Reducing the inflammation can prevent damage to the joints.
For systemic effects, the topical route is a promising alternative to medication delivery, and gels are regarded as the best transdermal delivery. The current endeavor aims to create Leflunomide Emulsomes for topical use. First, Emulsomes of Leflunomide were prepared by were prepared by thin film hydration method varied concentration of Drug, soya lecithin and cholesterol.

REFERENCE

[1] Lee J. E., I. J. Kim, M. S. Cho, and J. Lee, "A case of rheumatoid vasculitis involving hepatic artery in early rheumatoid arthritis," *Journal of Korean Medical Science*, vol. 32, no. 7, pp. 1207–1210, Jul. 2017.
[2] V. Picerno, F. Ferro, A. Adinolfi, E. Valentini, C. Tani, and A. Alunno, "One year in review: The pathogenesis of rheumatoid arthritis," Clinical

and Experimental Rheumatology, vol. 33, no. 4, pp. 551–558, Jul.–Aug. 2015.

[3] D. Piyarulli and R. M. Koolaee, "A 22-Year-Old Female with Joint Pain," in Medicine Morning Report: Beyond the Pearls, D. Piyarulli and R. M. Koolaee, Eds. Philadelphia, PA, USA: Elsevier, 2016, pp. 65–77.

[4] E. Smith, D. G. Hoy, M. Cross, T. Vos, M. Naghavi, R. Buchbinder, A. D. Woolf, and L. March, "The global burden of other musculoskeletal disorders: Estimates from the Global Burden of Disease 2010 study," Annals of the Rheumatic Diseases, vol. 73, no. 8, pp. 1462–1469, 2014.

[5] K. Mankia et al., "EULAR points to consider for conducting clinical trials and observational studies in individuals at risk of rheumatoid arthritis," Annals of the Rheumatic Diseases, vol. 80, no. 10, pp. 1286–1298, 2021.

[6] A. Struglics, S. Larsson, N. Kumahashi, R. Frobell, and L. S. Lohmander, "Changes in cytokines and aggrecan ARGS neoepitope in synovial fluid and serum and in C-terminal crosslinking telopeptide of type II collagen and N-terminal crosslinking telopeptide of type I collagen in urine over five years after anterior cruciate ligament rupture: An exploratory analysis in the Knee Anterior Cruciate Ligament, Nonsurgical Versus Surgical Treatment Trial," Arthritis & Rheumatology, vol. 67, no. 7, pp. 1816–1825, Jul. 2015.

[7] M. J. De Hair et al., "Features of the synovium of individuals at risk of developing rheumatoid arthritis: Implications for understanding preclinical rheumatoid arthritis," Arthritis & Rheumatology, vol. 66, no. 3, pp. 513–522, Mar. 2014.

[8] A. Mobashar, A. Shabbir, M. Shahzad, S. Hassan, et al., "Evaluation of immunomodulatory and antiarthritic potential of Trigonella gharuensis extracts," Evidence-Based Complementary and Alternative Medicine, vol. 2020, Art. no. 7, 2020.

[9] T. Jurca, L. Józsa, R. Suciu, A. Pallag, E. Marian, I. Bácskay, M. Mureșan, R. L. Stan, M. Cevei, F. Cioară, L. Vicaș, et al., "Formulation of topical dosage forms containing synthetic and natural anti-inflammatory agents for the treatment of rheumatoid arthritis," Molecules, vol. 26, no. 24, 2021.

[10] K. K. Sarkar, T. Mitra, R. N. Acharyya, et al., "Phytochemical screening and evaluation of the pharmacological activities of ethanolic extract of Argemone mexicana Linn. aerial parts," Oriental Pharmacy and Experimental Medicine, vol. 19, pp. 91–106, 2019.

[11] H. M. Wolff, "Future of transdermal drug delivery systems (TDDS)," American Pharmaceutical Review, Jun. 19, 2014.

[12] M. R. Prausnitz, S. Mitragotri, and R. Langer, "Current status and future potential of transdermal drug delivery," Nature Reviews Drug Discovery, vol. 3, pp. 115–124, 2004.

[13] M. A. Miller and E. Pisani, "The cost of unsafe injections," Bulletin of the World Health Organization, vol. 77, pp. 808–811, 1999.

[14] J. A. Bouwstra, P. L. Honeywell-Nguyen, G. S. Gooris, and M. Ponec, "Structure of the skin barrier and its modulation by vesicular formulations," Progress in Lipid Research, vol. 42, no. 1, pp. 1–36, Jan. 2003.

[15] G. W. Cleary, R. S. Lange, and D. L. Wise, "Medical application of controlled release," in Medical Application of Controlled Release, vol. 1. Boca Raton, FL, USA: CRC Press, 1984, pp. 203–245.

[16] S. Verma, A. K. Singh, and A. Mukherjee, "Formulation and evaluation of ketoconazole nanoemulgel," World Journal of Pharmacy and Pharmaceutical Sciences, vol. 5, no. 2, pp. 899–911, 2016.

[17] A. Pednekar, P. Dandagi, A. Gadad, and V. Mastiholimath, "Formulation and characterisation of meloxicam loaded emulgel for topical application," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 7, no. 11, pp. 216–222, 2015.

[18] A. Arien and B. Dupuy, "Encapsulation of calcitonin in liposomes depends on the vesicle preparation method," Journal of Microencapsulation, vol. 14, pp. 753–760, 1997.

[19] K. P. R. Chowdary and A. P. Kumar, "Recent research on formulation development of BCS class-II drugs: A review," International Research Journal of Pharmacy and Applied Sciences, vol. 3, no. 1, pp. 173–181, 2013.

[20] H. Eibl, "Synthesis of glycerophospholipids," Chemistry and Physics of Lipids, vol. 26, no. 4, pp. 405–429, 1980.