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Development and Evaluation of Methotrexate and Baicalin Loaded Nano-Lipid Carriers for Psoriasis Treatment

Short Title: Lipid Nano-Carriers for Psoriasis Treatment

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Abstract

Background: Psoriasis is a chronic inflammatory, T-lymphocytes immune-mediated skin disease. In this study skin-permeating nano lipid carriers (NLCs) of Methotrexate (MTX) and Baicalin was formulated. This further gave formulation scientists the possibility of encapsulating the existing potential drug moieties into nano-carriers, which when loaded into gels provided prolonged release and improved permeation.
Methodology: Optimization of formulation of NLCs were prepared and characterized by determination of their particle size, drug permeation, skin irritation, drug loading capacity, stability, in vitro drug release behavior and in vitro cellular viability. Ex vivo skin permeation and in vivo psoriatic efficiency were also evaluated and compared.
Results: Results revealed that dual drugs MTX amount permeating the skin was 2.4 to 4.4-times greater using single NLCs. The optimized dual drug-loaded NLCs had average particle size (150.20 ± 3.57 nm) and PDI (0.301 ± 0.01) and high entrapment (86.32 ± 2.78% w/w). The nanoparticles of MTX exhibiting a positive Zeta potential -38.6 mV. The PASI score obtained from skin irritation study revealed non-irritancy of the developed system. MT-BL NLCs was therefore able to inhibit the expression of inflammatory cytokines (TNF-α, and IL-17) to a greater extent.
Conclusion: It can be concluded that newer targeting strategies NLCs of dual drug delivery of nano-Lipid carriers that could be administered topically for the treatment of Psoriasis. Furthermore, this approach opens newer avenues for continued and sustained research in pharmaceuticals with much more effective outcomes.
Keywords: Psoriasis, Baicalin, Methotrexate, Nano structured Lipid Carriers, Topical delivery

Introduction

Psoriasis is a chronic inflammatory, T-lymphocytes immune-mediated skin disease characterized by deregulated multiplication of skin cells which increases its thickness causing appearance of salmon-red plaques with a silver scaly surface. The etiology of Psoriasis is still unknown. Several biochemical factors lead to maturation and proliferation of the epidermal cells (Chandra, Aggarwal, Manchanda, & Narula, 2019).Red and white/scaly patches are formed on the epidermis which is caused by immune system, the pathogens increase the epidermal growth and multiplication of epidermal cells (Srisuk, Thongnopnua, Raktanonchai, & Kanokpanont, 2012). Psoriasis is treated according to the severity of disease. Mild to moderate Psoriasis symptoms are treated topically while in severe disease systemic therapy and phototherapy is used. Systemic therapies are of major Concern throughout the past history. They are continuously developed and modified for the treatment of psoriasis (Ferreira et al., 2017). The first line treatment of Psoriasis is Methotrexate (Cytotoxic Drug) which is usually administer through oral and parenteral route Therefore transdermal and topical delivery of MTX with improved local and systemic delivery is prefer to reduced gastrointestinal side effects. (Abdelbary & AbouGhaly, 2015). Different methods for delivering MTX

topically to psoriasis lesions have been developed. (Srisuk et al., 2012). A traditional anti-psoriatic medicinal product, Methotrexate is most effect effective used as a single active ingredient or may use with combination of biologics (Uwe Wollina, Tirant, Vojvodic, & Lotti, 2019).MTX belongs to Dihydro-folate reductase enzyme inhibitor. ((Warren et al., 2016). This Drug shows good therapeutic activity in TNF (Tumor Necrosis Factor), Skin Tumor and Rheumatoid Arthritis. Due to high molecular weight of MTX which is 454.56 D, water solubility and the ionized form it will not diffuse passively through the Stratum Corneum (Al-Mahallawi, Fares, & Abd-Elsalam, 2019). Various types of MTX Based Drug Delivery System including Nano-Carriers, SLNs (Solid Lipid Nanoparticles), Self-emulsifying nano-systems, Transferosome's, Liposomes, Carbon Nanotubes, Polymeric nanoparticles, dendrimers, metallic nanoparticles, nano lipid carrier and niosomes formulated for the Topical Delivery of MTX(Al-Mahallawi et al., 2019). Baicalin (Scutellaria baicalensis) is a Traditional Chinese Herb, flavonoid extracted from the roots (Wu, Deng, Wang, & Li, 2020). S.baicalensis shows pharmacological activity against Psoriasis (Hung et al., 2018). Baicalin reduced the proliferation of keratinocytes and increased anti-tumor Activity (Wang et al., 2019).

The liposomes were introduced necessary to develop MTX entrapped liposomes. The Lipid carrier released the Drug before permeation into the target area(Malekar, 2014).These Deformable liposomes composed of Lipid content and a surfactant; an inner aqueous compartment surrounded by a Lipid bilayer formulated to increases the MTX skin penetration. The Advantage of Conventional liposomes over transfersomes is the characteristic of flexibility(Srisuk et al., 2012). A topical formulation of MTX and etanercept for the treatment of Psoriasis has previously reported and it gives a new pathway of combination formulations which noticeably increases the bioavailability and better skin permeation as compared to plain MTX gel. Dual drug therapy is the most frequent approach to treat psoriasis, it lowers drug systemic toxic effects, improves patient compliance and increase the efficacy of drug(Ferreira et al., 2017). Topical preparation of co-loaded Lipid Nano-Carrier Lipid soluble and water soluble Drug formulated(Lin, Huang, Zhuo, & Fang, 2010). The dual drug with different polarities are formulated with the aid of EA (Edge Activator) through the Film Hydration Method (TFH) (Sharma, Anandhakumar, & Sasidharan, 2015).

To achieve the treatment goal, it is then required to develop MTX-entrapped transfersomal formulations with improved permeability. Deformable transfersomes, elastic vesicles made of lipid materials and a surfactant with at least one inner aqueous compartment surrounded by a lipid bilayer known as transfersomes were introduced previously(Srisuk et al., 2012). Transfersomes are formulated from the Phosphatidylcholine (PC), Edge Activator Sodium Cholate (SC) and a surfactant KG Dipotassium Glycyrrhizinate for the entrapment of MTX. By using KG as a surfactant the amount of MTX permeated across the skin is 3-4 fold higher as compared to conventional liposomes(Rabia et al., 2020). Natural Ingredients based Transfersomes are the choice because of increase permeation of Drug into the skin(Srisuk et al., 2012).

No previous study of MT-BL co-loaded Nano-Lipid Carrier has been reported. They both have anti-psoriasis activity and have been used as a single drug carrier. This study aims to establish a Nano-Lipid Carrier containing two drugs and to evaluate their topical delivery for the topical treatment of psoriasis.

Material and Methods:

Materials:

Methotrexate was gifted from Werrick Pharmaceutical Islamabad, Pakistan. Tween 80 (Polysorbate 80) purchased by Bio-Labs from the source of HANGZHOU ZHONGBAO IMP & EXP CORP.LTD, China. CMC Sodium (Carboxy Methyl Cellulose) purchased by Bio-Labs from the source of QINGDAO ICD BIOCHEMISTRY CO.LTD. China. Sodium lauryl Sulphate purchased by Bio-Labs from the source of Emery Chemicals Malaysia. Soyabean (Phosphatidylcholine) PC purchased by Bio-Labs from the source of Vigilant Tenent Laboratories. Carbopol 940 purchased by Bio-Labs from the source of Lubrizol Advanced Materials INC. BRECKSVILLE USA. Carbopol 934 Bio-Labs from the source of Lubrizol Advanced Materials INC. BRECKSVILLE USA. Baicalin, and Sodium Cholate were purchased from Sigma Aldrich, USA. Phospholipon90G was received as a gift sample from Lipoid AG, Switzerland. PBS pH 7.4 and alamar Blue reagent were received from Thermo Fisher Scientific, USA. Cytokine standards IL-17 and TNF-α were purchased from BD Biosciences, California, USA. All other used reagents were of pure analytical grade.

Preparation of MT-BL TRS co-loaded TRs

Single MT-TRs and dual drug loaded MT-BL TRs were prepared by the thin film hydration method with some modifications (Chen et al., 2020). Phospholipon 90G, Tween 80 or Sodium Cholate as an Edge Activator and Methotrexate were dissolved in chloroform, methanol and HCl 1:1:0 at pH 3 mixture and evaporated at 50ºC by using rotary evaporator under vacuum at 90 rpm for 20 minutes. Thin film was evaporated under vacuum for removal of few traces of organic solvent. Dried film was hydrated with 100mg of Methotrexate (Srisuk et al., 2012)solution in 20ml PBS (pH7.4) for 1h at 60 ± 1º. The transfersomes were extruded 5 times of 2 minutes through

450 and 200nm filters. Dialysis was used for the purification of formulation from the unbound drug. The vesicles were stored at 4ºC in glass vials(Dar, Khalid, McElroy, Satoskar, & Khan, 2020).

Encapsulation of Baicalin:

A mixture of baicalin with cholesterol, chloroform and Tween 80 or Sodium Cholate was formed. Methotrexate loaded performed by using baicalin loaded Nano-Lipid Carriers in MT solution. Continued to stir 1mg/ml solution for 30 min. The excess drug present in supernatant was removed by washing with water. Stored the prepare MT-BL TRs at 4˚C in a dark place. The dual-drug loaded MT-BL/TRs were optimized in terms of entrapment efficiency (EE), Vesicle size (VS) and elasticity % by varying the percentage of PL OR SC.(Sharma et al., 2015).

Experimental Design for the Optimization of TRs:

The Nano-Lipid Carriers were prepared by using Thin Film Hydration Method through Rotary Vacuum Evaporator. Phospholipon 90G, Surfactant tween 80, Sodium Cholate and Cholesterol were added in methanol: chloroform (1:9) mixture of 10ml. In rotary vacuum evaporator film was allowed for 20 minutes at 50̊ C temp and RMP 90. The quantity of Phospholipon 90G and sodium cholate (PL:SC) ratio was varied as 90:10, 80:20, 70:30, 60:40, 80:20 and 80:20 and cholesterol were varied as 25 and 50mg for preparation of trial batches of Nano-Lipid Carriers formulations. The formulations were kept in desiccators overnight for the removal of the trace amount of Organic Solvents by evaporation. Dried film was hydrated with 100mg of Methotrexate solution in 20ml PBS (pH 7.4) for 1h at 60 ± 1º. The batches were termed as MT-BL TRs1, MT-BL TRs2, MT-BL TRs3, MT-BL TRs4, MTTRs and Blank TRs. Surfactant is selected on drug EE % and vesicle size of Nano-Lipid Carriers formed. Sodium Cholate lipid nano carrier formulation have higher flux value(Ita, Du Preez, du Plessis, Lane, & Hadgraft, 2007).

Physicochemical Characterization of co-loaded TRs:

Vesicle size, PDI and zeta potential of the prepared co-loaded TRs were measured using Zetasizer Nano ZS-90 instrument (Malvern instruments, Worcestershire, UK). All the batches were diluted with millipore water at 1:10 dilution and analyzed in triplicate using 90º scattering angle at 25ºC. Zeta potential was determined for drug loaded Nano-Lipid Carriers by Smoluchowski equation(Abdelbary & AbouGhaly, 2015). The EE % of Methotrexate was determined by direct method. Pellets obtained by centrifugation of Nano-Lipid Carriers for 15 mints at 15000 rpm. Then it was treated it with Triton X-100. Then 0.5ml methanol was added into the disrupted Nano-Lipid Carriers to make the drug more soluble. The sample was centrifuged at 10,000rpm for 5 minutes. (Doppalapudi, Jain, Chopra, & Khan, 2017)**.** Prepared the dilution 10ml of TRs and it was diluted up to 5ml with double distilled water. Un-entrapped MT was determined by direct method. In which the MT unentrapped separated from NLCs through exhaustive dialysis at 4ºC (Ferreira et al., 2017). Then MT-TRs added into the dialysis bag having Mol. Wt cut off 12-24 kDa containing PBS (pH 7.4) and stirred it into magnetic stirrer. Changed the PBS after every 2 h and determined its MT content through the AAS (Atomic Absorption Spectrophotometer). The sample was dissolved with Nitric Acid, heated it and Dried it (Dar, Din, & Khan, 2018).

Preparation of TRs gel:

The optimized TRs were loaded in 100mg of Carbopol 940 for topical preparation. The Carbopol powder was added in 10ml of distilled water and placed it in dark place for 24h. It swelled completely(Goyal et al., 2015).Drug loaded MT-BL TRs gel was formulated by adding 50 % (w/w) of MT/TRs and BL/TRs slowly in carbopol gel while constant stirring while simple MT/TRs gel was prepared by adding 10% (w/w) of single drug in the gel(Dar, McElroy, Khan, Satoskar, & Khan, 2020). Formulation was adjusted by neutralizing with Triethanolamine drop wise, a transparent gel was formed(Doppalapudi et al., 2017).

Nano-Lipid Carriers stability

The Optimized Transfersomal preparations were kept in storage at 4°C for 3 months. The evaluation parameters were Vesicle size, PDI, EE and zeta potential with different formulation's concentrations(Hsieh et al., 2021)

Deformability Index:

To find out the Deformability Index the developed TRs were formed from extrusion technique. The Vesicle size of TRs were find out earlier and later of extrusion Technique(Batool et al., 2021).

Physicochemical and Rheological evaluation of MT-BL TRs gel:

The MT-BL/TRs gel, MT-TRs gel and plain MTX gel were evaluated for pH, steady flow behavior, Thixotropy property, Visco-elastic behavior Gel measurements and water holding capacity.

Evaluation of pH

In 20ml of Distilled water add 1gm of each gel, pH of gel was determined by using a digital pH meter. A calibrated pH meter's electrode dipped in the dispersion medium of find out the pH of the gel.

Homogeneity

To a better patient's compliance, it is necessary to evaluate the homogeneity of topically applied transfersomal gel. Consistency of gel was measured by applying small quantity of gel at thumb and the Index Finger and rubbed these fingers over each other. Homogeneity was measured by its consistency.

Spreadability

A quantity of 0.5 g gel was placed between two transparent circular glass slides. Rest the gel over the glass for 5 minutes. The diameter was the indicator of measuring spreadability. Measured the diameter of the gel's circle.

Drug Content Determination

The MTX content was determined with Analytical method of MTX content (equivalent to 10mg) in a 100ml volumetric flask. Stirred the dilution and stand it upto 24hrs. Filtered the sample and analyzed it in AAS (Dar, Din, & Khan, 2018).

Rheological Studies:

Gel's viscosity was assessed by Brookfield viscometer. Spindle no 96 was used in viscometer to measure the flow behavior of gel. The sample was placed in the holder and spindle was attached with it and it was allowed to rotate at the speed of 5 rpm for 10-s run time at 37C to attain the minimum turning force of 10%. Various rpm speed used to determine the viscosity of gels.

In-Vitro Drug Release and Release Kinetic Study:

Franz diffusion Cell and dialysis membrane were method for in vitro drug release of drug for transfersomal dispersion. For activation of Dialysis membrane soaked it for 1 hr. PBS pH 7.4, sodium lauryl sulphate solution was the release medium. Filled the dialysis bag with 1ml of transfersomal formulation while release medium added into separate vial of 10ml.Placed it in shaker bath of shaking speed of 100rpm at 37˚C. Sample of 5ml was collected at a time interval of 0.5,1,3,6,24,48,96 and 120h and replaced it with 5ml fresh PBS medium. Analyzed the MTX contents by atomic absorption spectrophotometer (AAS), until no appearance of MT. Nitric acid was added after dialysis then heat for completely dried. HCl:Water 1:1 was added. Then it was boiled. Then best-fit model was used for regression co-efficient(Dar, Khalid, Varikuti, Satoskar, & Khan, 2020). The permeation flux study was performed for optimized transfersomal gel and plain gel. The slope of percentage of drug release v/s time is expressed for permeation flux (Walunj, Doppalapudi, Bulbake, & Khan, 2020).

In-Vivo Screening Model, CFA and Formaldehyde

For induction of psoriasis, a mixture of CFA and Formaldehyde (1:10 ratio) was prepared. Removed the hair from dorsal side of rats nearly 2*2 cm. A volume of 0.1 ml of prepare mixture was applied topically on the shaved area n=5 animals in each group at day 1, 2 and 3. Observed the psoriatic lesions, daily for 7 days.

(Srivastava, Nagar, Chandel, & Ranawat, 2016)

Anti-Psoriatic Activity of MT-BL TRs gel

Psoriasis was induced by the above mentioned method of CFA and formaldehyde. Animals were divided into 5 groups. 1. Disease untreated 2. Plain drug MT (water soluble) 3. Single loaded MT 4. Dual drug loaded MT-BL TRs treated every 24h for 21 days with MT TRs-gel (20mg/kg) and MT-BL TRs gel (20mg/kg) while the control group was left untreated. Drug efficacy was measured by PASI. The intensity of psoriasis was found by stain smears through microscopic examination(Otero et al., 2015).

Ex-Vivo permeation and drug deposition studies:

The Ex-vivo skin penetration studies were performed for all the trial batches using Dialysis Membrane and Franz Diffusion Cell. Firstly, abdominal hair of BALB/c mouse was removed using an animal hair clipper. The mice were then sacrificed; the skin samples and the abdominal fat tissues were excised. The excised skin was organized on the donor and receptor compartment with the SC side in the direction of the donor and dermis layers towards the receptor of the Franz diffusion cell apparatus. 7ml of PBS pH 7.4 was filled into the receptor compartment with a constant stirring rate of 300rpm at 32˚C. 1gm of the simple MT gel, single MT TRs or MT-BL TRs gel (equivalent to 20mg/kg and 5mg/kg of both drugs (MT-BL) was placed on the skin surface, The cumulative amount of MT and BL permeated were assessed by AAS Method, per unit area plotted against time(Dar, Khalid, McElroy, et al., 2020)Skin samples from the ex- vivo permeation study were saved and blot dried it. By using tape stripping method the stripped the skin pieces into 20 parts. Collected the entire tapes and placed them into the beaker. MT extracted when added tapes boiled in a mixture of HCL:Water (1:1). The remaining skin parts were chopped, meshed and homogenized(Doppalapudi et al., 2017).

Evaluation of skin Structure after MT-BL TRs gel Treatment

In-Vivo Skin irritation and histopathological study:

In histopathological study the epidermis changes and potential of irritation of psoriatic mice was used. The animals were divided into 5 groups with 5 animals in each group, group I had normal mice epidermis psoriasis was not induced to them, group II acted as a untreated control group, group III received Plain drug MTX, group IV received Single loaded MTX and group V received Dual drug loaded MT-BL TRs, respectively, applied topically for 1 week. Histopathological observation was performed to find the pathological changes during the topical application of gels. Prepared stripped skin samples from the sacrificed mice of different treatment groups. Stained the skin samples with H & E and cryostat microtome on slide and observe under electric light microscope(Abdelbary & AbouGhaly, 2015).

Macrophage cytotoxic assay:

Several cytokines are involved in the regulation of immunity against psoriasis. The IL-17 mainly produced by Th-17 Cells. The IL-17 has important role in the production chemokine and secretions of neutrophils and anti-microbial proteins at the site of inflammation. In psoriatic skin samples the cytokines levels (TNF-α and IL-17) were determined by ELISA (Enzyme-linked immunosorbent assay)(Babaloo, Oskoei, Kohansal, Barac, & Ahmadpour, 2020).Skin tissues of induced psoriasis treated with PBS and then the mixture was properly homogenized in a tissue homogenizer at 3000rpm for 5 minutes. After centrifugation at 10,000 rpm for 15 minutes at 4˚C, the levels of TNF-α and IL-17 was determined by ELISA according to manufacturer's protocol(Jain et al., 2017).

In-Vivo efficacy of formulation in BALB/c infection model of Psoriasis:

Clinical severity was expressed by the PASI (Psoriasis Skin Area and Severity Index). It developed on the basis of psoriasis affected area and severity index. Redness, scales and erythema was scored independently on a scale from 0 to 4: 0 none; 1 slight; 2 moderate; 3 marked and 4 marked. PASI was calculated on the basis of redness, erythema and scales. Anesthesia was given at the end and the sample of skin was collected. Preserved in 10% formalin solution for histological examination. Stained the rat's skin specimen in hematoxylin and eosin dye for histological examination(Walunj et al., 2020).

Statistical Analysis:

The analysis of trial batches of MT-BL TRS gel was assessed by response surface methodology method. The assessment responses were analyzed by surface plots and contour plots to observe the design space to find out the suitable quantities of excipients for maximum responses. Optimization plot were explained formulation factors and levels which produced the desired target responses. One way ANOVA test applied for the comparison between groups. For significant P value, multiple tukey tests were used to compare the means of different groups. The significance level in this study is 0.05. SPSS V23 software was used. Kruskal-Wallis test for non-parametric statistical differences was used(Dar, Din, & Khan, 2018).

Results

The optimized dual drug formulation were characterized on the basis of Physio- chemical parameters include the varying Concentration of surfactant and Edge activator, Vesicle size, Polydispersity Index, Entrapment Efficiency and *In-Vitro* and *Ex-Vivo* Drug Permeation study.

Selection of Surfactant and edge Activator:

For the flexibility of MT-BLTRs various Edge Activators were studied such as Sodium Cholate, Tween 80, Phospholipon 90G, Sorbitan monolaurate, Sorbitan monopalmitate, Sorbitan Stearate and Sorbitan monolaurate(Y. Singh et al., 2017). The Sodium Cholate was selected on the basis of observations seen in dispersion, it increased the flexibility of Vesicle while with other Surfactants the Frothing has been seen in Dispersion.

Formulation of Nano-Lipid Carriers

Total of six formulations were formulated with different concentration of Phospholipon 90G and Sodium Cholate. The Transfersomal preparations were prepared using 'Thin Film Hydration Method'(Dar, McElroy, et al., 2020). After formulation of Dual drug loaded carbopol gel TRs was evaluated on the basis of the key parameters i.e. Vesicle Size, PDI, Deformability Index and Entrapment Efficiency. The Optimized Formulation Code with MT-BL TRs 4 were selected for further study.

Physiochemical characterization of NLCs

MT-BL TRs were prepared by Thin Film Hydration Method, Sodium Cholate is used as an Edge Activator. Co-delivery of MT-BL TRs will allow a targeted delivery of nanoparticles to the immune system involved in the Psoriasis pathology.

The main results of physiochemical properties are shown in table 1. These shows that mean Vesicle size decreases with the increases in the SC with a quick reduction in vesicle size as SC reached 10%. It was established that the vesicle size of single MTTRs was considerably higher ($p<0.01$) at 10% of SC as compared to MT-BLTRs. In table 1 the PDI value is <0.3 of formulations containing EA 5% and 10%. This results in homogenous dispersion. When the SC % is increasing from 10% to 20%, the PDI shows increase in its value.
MT-BL TRS incorporation did not affect the average size of TRs. The TRs had a PDI in the range of 0.116 (Blank TRs) to 0.359 (MT-BL TRs). The PDI values suggest that the transfersomal preparation is homogenous with low tendency for aggregation. The average vesicle size of MT-BL TRs was 170.1 ± 3.7nm with a PDI value of 0.138 and a ZP value of -38.6 Fig 1. The PDI value of MT-TRs and MT-BL TRS ranges from 0.15 to 0.359 which shows better uniformity and homogeneity of formulations. Deformability Index is the major parameter of NLCs for topical drug delivery due to which drug molecules easily permeate into the skin with the help of Edge activator(Batool et al., 2021).

Moreover, the effect of SC on the deformability index was increasing with each other at the extent of 10% ($p < 0.01$) the deformability index was decreased. The values of deformability index of Blank TRs, single MTTRs, and MT-BL TRs were 59.7±3.7, 56.1 ± 3.3 and 52.8 ± 2.4, respectively. This explained that by adding of Baicalin created a detrimental influence on the deformability of elastic vesicles on the other hand the addition of MTX had optimistic effect. It was observed that the lipid content increases the particle size of Nano-Lipid Carriers.

Optimization

Formulation code	PL:SC	Vesicle size (nm)	PDI	Deformability index	EE% ± SD (PT/CR)
MT-BL TRs 1	90:10	241.5 ± 7.4	0.154	45.3 ± 2.7	71.9 ± 5.2/86.8 ± 5.7
MT-BL TRs 2	80:20	170.1 ± 3.7	0.138	52.8 ± 2.4	69.5 ± 4.7/81.9 ± 4.4
MT-BL TRs 3	70:30	111.3 ± 4.1	0.272	49.7 ± 2.9	47.0 ± 4.1/59.7 ± 4.8
MT-BL TRs 4	60:40	56.4 ± 3.5	0.359	33.5 ± 2.6	37.8 ± 5.0/44.1 ± 4.9
MT TRs	80:20	152.5 ± 5.0	0.131	56.1 ± 3.3	33.7 ± 4.1
Blank TRs	80:20	141.2 ± 3.5	0.116	59.5 ± 3.7	-

PL: Phospholipon 90G; SC: Sodium cholate; PDI: Polydispersity index; EE: Entrapment efficiency; MT-BL TRs: Methotrexate-baicalin dual loaded transfersomes; MT TRs: Single methotrexate loaded transfersomes

Table 1 Physio Chemical Characterization

Effect of independent variables on Vesicle size
The Vesicle Size of the optimized formulation of Co-loaded Nano-Lipid Carriers were evaluated using Zetasizer Nano ZS-90 instrument (Malvern instruments, Worcestershire, UK) The varying Concentration of PL:SC have significant effect on dual drug loaded transfersomal preparations. The vesicle size of formulations is shown in table 1. There is no considerable difference in vesicle size in 70:30 or above concentrations but at 60:40 the rapid reduction dual drug loaded MT-BL TRs has been observed. Preferred vesicle size was obtained by a sonication of 10 minutes.
Effect of Independent variable on Zeta Potential
Vesicle's charge is evaluated by the Zeta potential. The findings of zeta potential for the single MTTRs and MT-BL/TRs are described in table 1. The result of zeta potential is around -38.6 mV which is considered as stable colloidal dispersion. MT-BL TRs incorporation did not have significant interference in the Zeta Potential values & consequently in the stability of formulations. A neutral

charged or a slightly negative Nano-Lipid Carriers with a zeta potential ranges from -10mV to +10mV are acceptable.

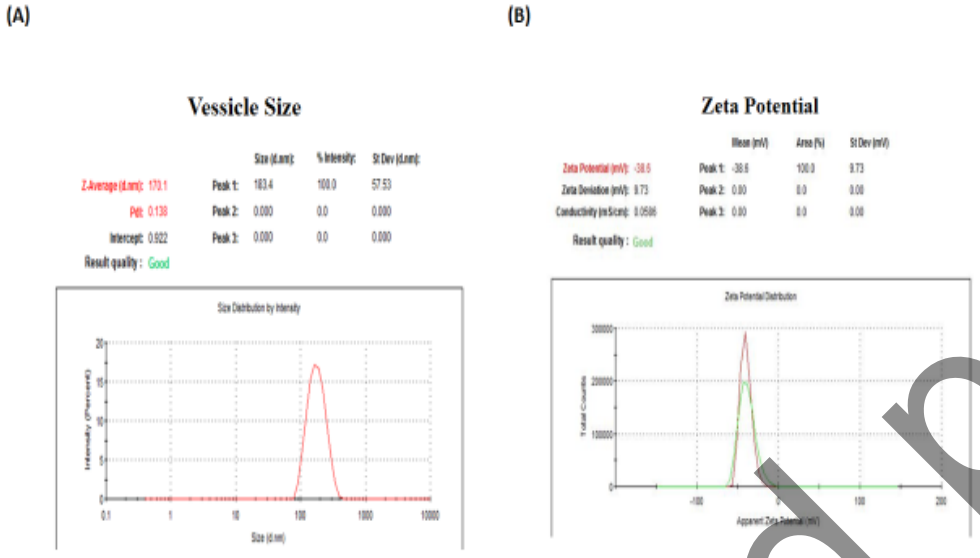


Figure 1. Independent Variables

Effect of Independent variable on percentage entrapment efficiency

The Entrapment Efficiency (EE) of TRs were evaluated (Table 1). It was observed that gradual decreased in EE with a increase of PL:SC (Phospholipon 90G: Sodium Cholate) until 10%. More increases in PL:SC resulted in an immediate decrease in EE. Single MTTRs have lower EE as compared to dual drug loaded MT-BL TRs. After consideration of all the important factors the Methotrexate co-loaded Nano-Lipid Carriers having PL:SC of 60:40 and vesicle size 56.4±3.5 nm were selected for the rest of the studies because it shows a high EE. Dual drug loaded TRs shows increase in EE as compared to single loaded hydrophilic drug.

The EE % of MT-TRs was about 37.8% and a Blank 33.7% which indicates the further incorporation of Drug didn't affected by the functionalization process. The EE% of the results are relatively high which shows that the transfersomal preparation has a better stability and good entrapment of a drug.

Physiochemical &Rheological evaluation of TRs gel

Spreadabilty:

MT-BL TRs were formulated with the Carbopol 940 gel base which retained the Drug's Concentration for the prolong period of time into the Stratum Corneum. The spreadability factor of TRs gel was evaluated for the characteristics of Topical gel formulation. Table 2 shows the spreadability profiles of MT-BLTRs gel, MT-TRs and Blank TRs. No significant difference in their physiochemical properties has been observed in all transfersomal carbopol gels. At initial stages the spreadability profile of all Transfersomal gels were of similar results.

MT-BL-TRs gel was evaluated for rheological behavior. The Rheological properties of transfersomal gel was analyzed for topical application. The results were compared with the blank drug gel. The viscosity of gels were analyzed by Brookfield viscometer spindle no 96.

Dual loaded drug incorporated gel showed shear thinning characteristics by applying a slightest shear stress explained the pseudoplastic behavior. This assumes that physical stability of the formulations under several conditions during manufacturing and transportation.

Formulation Code	pH	Homogeneity	Spreadability	Drug content Determination	Rheological studies
Formulation 1	6.1	1.32	1.42	2.002	20.18
Formulation 2	6.3	1.36	1.32	1.996	22.92
Formulation 3	6.5	1.34	1.46	2.005	20.46
Formulation 4	6	1.29	1.48	2.321	22.48
Formulation 5	6.2	1.34	1.35	2.505	21.87
Formulation 6	5.9	1.35	1.30	1.882	18.44

Table 2 Rheological Properties

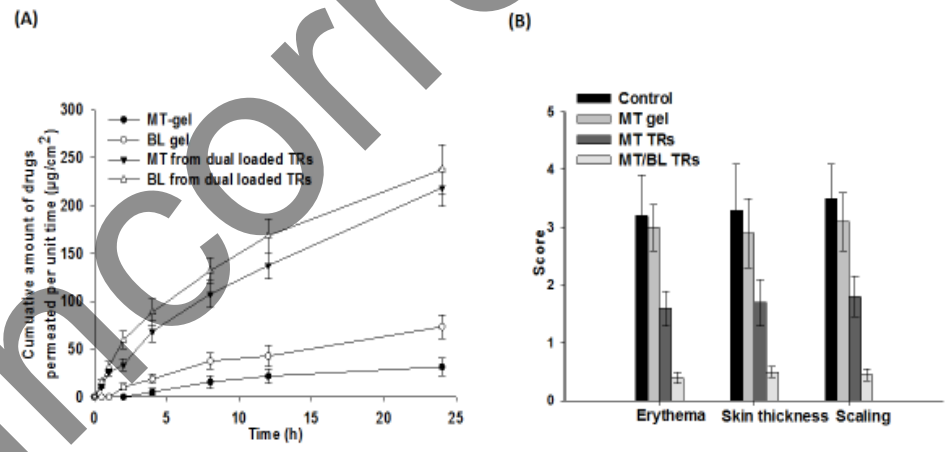
Ex-Vivo Skin Penetration

In-Vitro and Ex-Vivo permeation studies were performed as per the experimental protocols was approved by ethical committee of University of xxx. The studies were carried out for all the transfersomal batches for determination of Drug release and permeation studies. The permeation flux for the trial batches of transfersomal gel and plain drug were determined.

Methotrexate 20mg/kg (Srisuk et al., 2012) and Baicalin 5 mg/kg (García-Manrique et al., 2020)

The outcomes of applying (Methotrexate)MT 20mg/kg and Baicalin 5mg/kg of formulation on the normal mice skin, have shown no MT and BL in the acceptor compartment within 24 h but the same dosage of MT and BL applied on psoriatic skin, amount of 50% penetration is detected in the acceptor compartment. The total amount permeated per unit area from simple MT gel, Single MTTRs gel 31.42 µg/ml was released with SD± 9.4 µg/cm2, Single BLTRs gel 73.2 µg/ml was released with SD± 12.4 µg/cm2. In combination drug delivery the MT-BL TRs the MT was 218.6 µg/ml permeated with SD ±19.5 µg/cm2 and the BL was 237.61 µg/ml permeated with SD ±25.5 µg/cm2 respectively. When drugs were applied with co-loaded MT-BL TRs the skin permeation of drugs was much improved ($p<0.01$). However co-loaded MT-BL TRs when applied in the skin it was more efficiently deposited as compared to a simple MT and single MT gels.

Important parameters of study are summarized in the results. The Vesicle Size and lipid content behavior of Nano-Lipid Carriers (liposomal formulations) affected the release pattern of TRs. Additionally, the MT-BL TRs deposition in the skin is much higher than the single drug loaded MT gel. It has more prolong retained period of time at the site of psoriasis due to more skin deposition as compared to less skin permeation (Dar, McElroy, et al., 2020).



MT-gel: Plain Methotrexate gel; BL gel: Plain Baicalin gel; MT from dual loaded TRs: Methotrexate dual loaded transfersomes; BL from dual loaded TRs: Baicalin dual loaded transfersomes.

Figure 2 Drug's Permeation and Skin Penetration studies

Evaluation of Skin Structure after MT-BL TRs treatment:
Histopathological examinations compared the healthy mice normal skin having typical epidermis and dermis with psoriatic induced treated or untreated mice. The results obtained from group I (normal mice epidermis) Fig 2A, group II (untreated control) Fig 2B, group III (Plain drug MT) Fig 2C, group IV (Single loaded MT) Fig 2D and group V(Dual drug loaded MT-BL TRs)Fig 2E, respectively. The group I illustrated normal skin with well-defined epidermis, dermis, subcutaneous tissue and muscles. The epidermis revealed stratified squamous keratinized epithelium that was supported by a dermis layer of dense fibro elastic connective tissue that was devoid of any inflammatory cells. The histopathological changes of treatment group were highly dependent on formulation type. The Thickness index varies in all groups. During the treatment the thickness was reduced. After treated with single MT Gel, psoriatic skin illustrated insignificant reduction in the thickness of epidermis. This result recommended that the anti-psoriatic activity of Single MT Gel was relatively partial. In contrast, single MT-TRs showed a better anti-psoriatic activity by reducing thickness of epidermis which indicates that Nano-Lipid Carriers of MT within Nano-Lipid Carriers have better anti-psoriatic activity but MT-BL TRs a dual drug delivery gel showed similar histopathological characteristics as compared to the normal epidermis of mice, the epidermis of the skin was almost normalized. This confirms that single MT-TRs and MT-BL Nano-Lipid Carriers have significant ($p<0.05$) reduced thickness in all the groups. Relatively acceptable safety profile and doesn't cause irritation and in clinical trials when applied topically. This was based on that there were no apparent signs of skin irritation in the conducted in-vivo study.

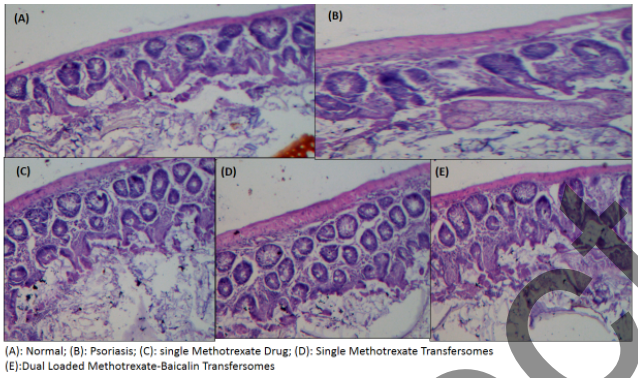


Figure 3 Histopathological Studies

Scoring of Skin Inflammation:
Effect of formulations on anti-psoriatic effectiveness was evaluated by PASI scoring of skin's severity and thickening. For consecutive 30 days the PASI scores of skin erythema and skin thickening in psoriasis affected area were observed. All formulations of simple MT drug, single MT-TRs and MT-BL TRs exhibited clear PASI scores at day 10 which was later more improved on day 15. After compared to these formulations the dual drug loaded MT-BL TRs gel showed reduction in both skin erythema and skin thickening and has the best anti-psoriatic activity. The PASI score calculated as 0 to 6, the scoring parameters are 1. Erythema 2. Skin Thickness 3. Scaling. The formulations for PASI scoring were Control group, simple MTX gel, MTX/TRs Gel, MTX/TRs gel and MTX/BL TRs gel. Control group showed score 3 in Erythema, skin thickness and scaling. Control group showed the highest score in all the parameters which shows that it has the severe erythema, skin thickness and scaling. Simple MTX gel: showed less score of 3 as compared to Control Group for all the parameters Erythema, skin thickness and scaling. Simple MT gel showed the score of 2.5 for all the parameters which shows that the severity of erythema, skin thickness and scaling going to decreased.MT/TRs gel: The scoring scale has shown decrease up to 1.5 in PASI scoring. The TRs gel preparation has more efficacy and permeation into the skin as compared to plain MT gel. MT/BL TRs gel: The combination dual drug delivery has shown the scaling of zero or above for all parameters which shows that the dual drug combination of MT/BL TRs gel has the lowest erythema, skin thickness and scaling which leads to improve the improve the condition of Psoriasis.

Cytotoxicity Assay:
By increased level of cytokines, psoriatic skin is characterized. TNF-α and IL-17(Pietrzak et al., 2008). ELISA assay was performed to find the level of TNF-α and IL-17 as shown in Fig 4A and Fig 4B. The TNF-α and IL-17 were analyzed in 5 groups. Each group consisted of 5 members. The optimized topical

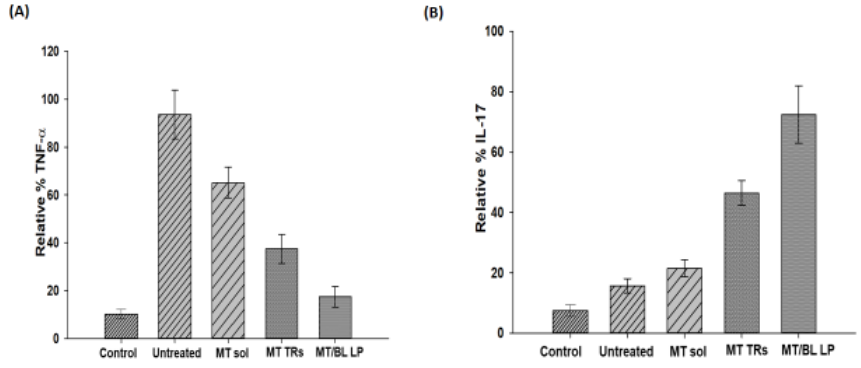
transfersomal gel for psoriasis decreased the level of cytokines TNF-α, Interleukin-22 and Interleukin - 17(Doppalapudi et al., 2017).

TNF-α:

Fig 4A showed the results of relative % of TNF- α at a scale of 0-120 at Y-axis and different group formulation of Control, Untreated, MT sol, MT TRs and MT/BL TRs. The results are showing major differences in Control and untreated group. The % of TNF-α increased upto 90%, Simple MT sol: showed 65% TNF-α. The relative % of TNF- α decreased as compared to the untreated group. Methotrexate is effective in psoriasis as the results showed the decrease value. As the MT/TRs gel is incorporated with Methotrexate. Gel has more penetration permeation as compared to the plain MT Sol. The TNF –α was decreased upto 35%. The MT/BL TRs gel % relative of TNF-α has been 10% which shows near 5% of control group. The results were interpreted as the Nano-Lipid Carriers has more permeation and penetration as compared to the plain gel. The transfersomal gel has more efficacy as compared to plain single drug delivery. The results are similar with control group it shows the better choice of Nano-Lipid Carriers in topical drug delivery. The results of control group is much concise as compared to other groups. The control group was used as a standard and the relative % of TNF-α was 5 %. Other groups have high relative % of TNF-α, the untreated group have the highest % of TNF-α.

IL-17

The Relative % IL-17 were significantly higher in patients with Psoriasis as compared to Control Group. As it has been treated with different formulations the relative % of IL-17 was twice times higher with other formulations. Fig 4B showed the results of relative % of TNF- α at a scale of 0-120 at Y-axis and different group formulation of Control, Untreated, MT sol, MT TRs and MT/BL TRs. The results are showing major differences in Control and untreated group. The % of IL-17 increased up to 65%, Simple MT solution showed 20% IL-17. The relative % of IL-17 increased as compared to the untreated group. Methotrexate is effective in psoriasis as the results showed the increase value. As the MT/TRs gel is incorporated with Methotrexate. Gel has more penetration permeation as compared to the plain MT Sol. The IL-17 was increased upto 40%. The MT/BL TRs gel % relative of IL-17 has been 65% which shows far away from control group. The results were interpreted as the Nano-Lipid Carriers has more permeation and penetration as compared to the plain gel. The transfersomal gel has more efficacy as compared to plain single drug delivery. The results are similar with control group it shows the better choice of Nano-Lipid Carriers in topical drug delivery. The results of control group are much concise as compared to other groups. The control group was used as a standard and the relative % of IL-17 was 20 %. Other groups have high relative % of IL-17 the untreated group have the lowest % of IL-17. The comparison between TNF-α and IL-17. In comparison with the control group the TNF-α and IL-17 was relative of 20.In untreated group the level of TNF-α were increased upto 90 % in psoriatic induced untreated skin, respectively. After application of MT-soln, MT- TRs and MT/BL TRs the level of TNF-α was decreased by 65%, 25%, 20%. Similarly in untreated group, the level of IL-17 was raised in comparison to control group which signifying the induction of psoriasis (Fig 2). After application of MT-soln, MT- TRs and MT/BL LP the level of IL-17was increased by 20%,40% and 65%. The present data has shown that increased serum concentration of IL-17 is present in the serum concentration. The role of IL-17 in the production of neutrophil at the site of inflammation and chemokine production. The TNF-α cytokine Assay data shows that in Psoriasis affected group (un treated) the serum level was increased which was later on decreasing when treated with Methotrexate Nano-Lipid Carriers. The IL-17 Cytokine Assay data shows that in Psoriasis affected group (un treated) the serum level was decreased which was later on increasing when treated with Methotrexate Nano-Lipid Carriers. Both Cytokines assays showed the activity of Nano-Lipid Carriers of MTX Soln, MT-TRs and MT/BL TRs. TNF-α interacts with inflammatory cells to trigger cytolysis.



MT sol: Methotrexate solution; MT TRs: Methotrexate Transfersomes; MT/BL LP: Methotrexate Baicalin Liposomes.

Discussion:

In this study, the Dual drug nano carriers Physio-Chemical and *In-Vitro, In-Vivo* characteristics were examined. The topical treatment of psoriasis is preferred over systemic drug delivery due to fewer adverse effects(Rahman et al., 2012). The topical formulations have more bioavailability with the fewer side effects. The dual drug delivery shows a significant therapeutic approach at a very lower dose for the complicated and single therapy resistant diseases(Dar, Khalid, McElroy, et al., 2020). Adverse Drug Reaction were reported with Systemic Methotrexate Therapy in Psoriasis Patients but there was no significant effect on Liver and Serum Enzyme Level(U Wollina, Ständer, & Barta, 2001) .To improve the efficacy of treatment of various pathological diseases dual drug therapy is required. The limitations of dual drug delivery method are Entrapment of different charged molecules, Physio-chemical incompatibility, solubility, selection of surfactants, stability and various drug concentrations.(Malekar, 2014). Co-loaded Nano-lipid carrier penetrated and permeated into the deeper layer of dermis, slowly releasing a dual drug into the subcutaneous(Cosco et al., 2015). Previously, many studies have described the Methotrexate nano-carrier Skin penetration results for the topical application for the treatment of Psoriasis(Rashid et al., 2021).It was concluded that the VS of single MTTRs was considerably higher ($P<0.01$) at 10% of Sodium Cholate as an EA compared to MT-BLTRs.

Edge Activator act as a stabilizer to increase the Drug Permeation of Lipid- nano carriers(Al-Mahallawi et al., 2019). The VS decrease with the increase of Sodium Cholate increases 10% to 20%, the PDI shows increase in its value. The PDI value of MT-TRs and MT-BLTRs ranges from 0.15 to 0.359 which shows better uniformity and homogeneity of formulations(Qushawy, Nasr, Abd-Alhaseeb, & Swidan, 2018).The hydrophobic drug have the higher membrane flux and have the direct interaction with the lipid bi-Layer of the Skin. Phospholipon 90 G increased the permeation flux ($P<0.05$) of MTX formulation as it has the high lipid content. The effect of Sodium Cholate on the Deformability Index was increasing with each other at the extent of 10% ($P<0.01$). The amount of EE was significantly decreased when the amount of surfactant increased(Khan et al., 2015). The Nano-Lipid Carriers prepared by the Sodium Cholate has the concise particle size and EE% is high as compared to Tween 80. Sodium Cholate gave better Entrapment efficiency as compared to other surfactants. The formulation of MTX, Phospholipon 90G, Sodium Cholate and Cholesterol has the most concise particle size and better Entrapment efficiency.In TRs vesicles the more lipid content the more of its EE. The effect of Cholesterol in formulation is least effective but the effect on EE is more due to its high lipid content. Phospholipon 90G has the greater influence on EE %. This explained that by adding of Baicalin created detrimental influences on the deformability of elastic vesicles on the other hand the addition of MTX had optimistic effect.To increase the deformability of Ultra-deformable liposomes the EA are of vital importance.The effect of Sodium Cholate as an Edge Activator is concentration-Dependent(Yang et al., 2019). The advanced rigid molecular structure have the more Skin permeation, rigid vesicle size and more bioavailability(Chaudhary, Kohli, & Kumar, 2013). In the present study, Single MTTRs have lower EE as compared to dual drug loaded MT-BL TRs. The decrease concentration of EA and Phospholipon 90G decreases the rapid reduction of Dual Drug Loaded TRs Vesicle Size. More increase of Phospholipon 90G and Sodium Cholate resulted in immediate decrease in EE.Single MTTRs have lower EE as compared to dual drug loaded MT-BL TRs.Dual drug loaded TRs shows increase in EE as compared to single loaded hydrophilic drug. The transfersomal preparations have a better stability and good entrapment of a drug. Vesicle size of Nano-Lipid Carriers has an important role on topical delivery. To achieve the advance the targeted

drug delivery and deeper penetration of drug the formulation should be optimum and characterized on the basis of particle size. These formulations varying from different (Phospholipon 90 G and sodium cholate) concentrations were prepared by Thin film hydration method.Polar and high molecular weight molecule diffused through Stratum Corneum by encapsulation with non-ionic surfactant of particle size(García-Manrique et al., 2020). To increase the stability of formulation the Vesicle should be a highly negative zeta potential charge due to electrostatic repulsion. The highly positive charged nanoparticles are more cytotoxic as they caused protein aggregation in blood.To find out the stability, cellular uptake and cyto-toxicity of transfersomal preparation the Zeta potential is required(Chandra et al., 2019).The stable colloidal dispersion exhibited a zeta potential is around -38.6 mV. The highly positive charged nano particles are more cytotoxic as they cause protein aggregation in the blood. The surface charge of Nano-Lipid Carriers maintains its stability(Doppalapudi et al., 2017). The Ideal pH for carbopol gel is 5.0-8.0 which does not affect its Rheological properties and used as a topical formulations(Roh et al., 2015)**.** The formulations of carbopol gel having different Concentrations shown a Non-Newtonian, higher shear-thinning which increased the Drug's Retention time, bioavailability and its therapeutic efficacy (Dar, Khalid, McElroy, et al., 2020)**.** Nano-carrier emulsion gel are more beneficial in topical preparations hence they covered the maximum coverage area(Kaur, Jain, & Singh, 2015).The dual drug delivery of MTX and Baicalin into the nano-carrier molecule improves the therapeutic activity as compared to single drug delivery. The Previous studies has been reported(Cosco et al., 2015).The Thin Film was formed at 50°C Temp and 90 RPM at 20minutes in Rotary Vacuum Evaporator. Nano-lipid carriers have 5 fold more penetration of drug into the Stratum Corneum. (Doppalapudi et al., 2017). Lipid nano-carrier is the advanced Drug Delivery System in Cosmeceuticals. The Novel Drug Delivery system termed as "Nano-Safe Carriers" due to their safety profile.(Puglia & Bonina, 2012)**.** The components are chosen on the characteristics of skin permeation, Molecular Compatibility and GRAS condition(Kaur et al., 2015)**.** In Previous study, the dual drug delivery of MTX lipid ultra-deformable liposomes formed by a carbopol gel which shows increased skin drug Bioavailability(Ferreira et al., 2017). For the Topical route of Drug administration the spreadability is the important characteristics for the development and formulation of appropriate Drug into the target area. The PSRAL Gel and PSRCL Gel showed the rheological properties of gels result in reduction of viscosity due to shear stress(Pradhan et al., 2018).Drug loaded incorporated gel showed shear thinning characteristics by applying a slightest shear stress explained the Pseudoplastic behavior. This shows the stability of the formulations. *In-Vitro* and *Ex-Vivo* permeation studies of all the transfersomal batches for determination of Drug release and permeation studies. The MT-BLTRs the skin permeation of drugs was much improved ($P<0.01$) However co-loaded MT-BLTRs when applied into the skin it was more efficiently deposited as compared to a simple MT and single MT gel. The vesicle size and lipid content behavior of TRs affected the release pattern of TRs. It has more prolong retained period of time at the site of psoriasis due to more skin deposition. *In-Vitro* results of MTX SLNs showed a 8 hrs sustained release*(Ferreira et al., 2017).*The dual drug MTX loaded TRs decreased the PASI score, the formulation was developed for treating Psoriasis topically(Chandra et al., 2019). *In-Vivo* results explained the lipid nano-carrier anti-psoriatic activity, decreased the Interleukin-17 and Tumor Necrosis Factor-α. Cell lines explained the decreased level of NO, Interleukin-2, Interleukin-6 and Interleukin 1β(Jain et al., 2017).

Methotrexate with baicalin transfersomal gel preparation showed a significant penetration and permeation parameters in psoriasis affected skin. Psoriasis is a chronic inflammatory, T-lymphocytes immune-mediated skin disease. The etiology of psoriasis is yet unknown but the risk factors are Drugs associated, IBD Disease, life style, environmental and genetic factor which leads to the proliferation of keratinocytes(Sala, Elaissari, & Fessi, 2016). As a result Silver scales, Papules and plaques are formed due to the epidermal thickening. The scaly skin lesions usually be observed at elbows, knees joints, palms, soles, extensor surfaces and the Erythrodermic psoriasis diffuse lesions covering >90% body surface. Psoriasis treatment focuses on relieving symptoms and improving skin function. Depending upon the type and severity of psoriasis the treatment should be planned it may be Phototherapy Treatment, Systemic treatments, Monoclonal antibodies, Topical treatments(S. Singh et al., 2016).Application of Drug Directly at topical affected psoriasis site with narrow therapeutic window reduced the systemic absorption adverse effects (How, Yap, Lim, Goh, & Lai, 2020). Methotrexate (Orally as well as systematically) is the gold standard Drug for the treatment of Psoriasis. MTX belongs to Dihydro-folate reductase enzyme inhibitor. This Drug shows good therapeutic activity in TNF (Tumor Necrosis Factor), Skin Tumor and Rheumatoid Arthritis. Due to high molecular weight of MTX which is 454.56 D, water solubility and the ionized form it will not diffuse passively through the Stratum Corneum(Tan, Liu, Guo, & Zhai, 2011). Various types of MTX Based Drug Delivery System including Nano-Carriers, SLNs (Solid Lipid Nanoparticles), Self-emulsifying nano-systems, Nano-Lipid Carriers, Liposomes,

Carbon Nanotubes, Polymeric nanoparticles, dendrimers, metallic nanoparticles, nano lipid carrier and niosomes formulated for the Topical Delivery of MTX(Trotta, Peira, Carlotti, & Gallarate, 2004). MTX-entrapped Nano-Lipid Carriers are formulated elastic vesicles made of lipid materials and a surfactant with at least one inner aqueous compartment surrounded by a lipid bilayer(Rai, Pandey, & Rai, 2017). By using KG as a surfactant the amount of MTX permeated across the skin is 3-4 fold higher as compared to conventional liposomes. Natural Ingredients based Nano-Lipid Carriers are the better choice because of increase permeation of Drug into the skin. Formulations were Analyzed and optimization by thin film rehydration using Phospholipon 90G, Tween 80 and Cholesterol. Optimization and characterization of drug carriers based on particles size, zeta potential and drug entrapment efficiency. Evaluation of pH, Homogeneity, Spreadability, Rheological Studies & Drug content Determination for all the formulations it was observed that drug loaded TRs (MT-BL/TRs) have better physiochemical properties as compared to plain Drug.

The result of vesicle size in 70:30 or above concentrations but at 60:40 the rapid reduction dual drug loaded MT-BL TRs has been observed. Zeta potential is around -38.6 mV which is considered as stable colloidal dispersion. MT-BL TRs incorporation did not have significant interference in the Zeta Potential values & consequently in the stability of formulations. A neutral charged or a slightly negative Nano-Lipid Carriers with zeta potential ranges from -10mV to +10mV are acceptable. The highly positive charged nanoparticles are more cytotoxic as they caused protein aggregation in blood. The EE % of MT-TRs was about 37.8% and a Blank 33.7% which indicates the further incorporation of Drug didn't affect by the functionalization process. The EE% of the results are relatively high which shows that the transfersomal preparation has a better stability and good entrapment of a drug. Dual loaded drug incorporated gel showed shear thinning characteristics by applying a slightest shear stress explained the pseudoplastic behavior. This assumes that physical stability of the formulations under several conditions during manufacturing and transportation.

Characterization by In-vitro release and membrane diffusion studies of transfersomal gel formulation, the Vesicle Size and lipid content behavior of Nano-Lipid Carriers (liposomal formulations) affected the release pattern of TRs. Additionally, the MT-BL TRs deposition in the skin is much higher than the single drug loaded MT gel. It has more prolong retained period of time at the site of psoriasis due to more skin deposition as compared to less skin permeation.

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Histopathological examinations compared the healthy mice normal skin having typical epidermis and dermis with psoriatic induced treated or untreated mice. The epidermis revealed stratified squamous keratinized epithelium that was supported by a dermis layer of dense fibroelastic connective tissue that was devoid of any inflammatory cells. The histopathological changes of treatment group were highly dependent on formulation type. The Thickness index varies in all groups. During the treatment the thickness was reduced. After treated with single MT Gel, psoriatic skin illustrated insignificant reduction in the thickness of epidermis. This result recommended that the anti-psoriatic activity of Single MT Gel was relatively partial. In contrast, single MT-TRs showed a more better anti-psoriatic activity by reducing thickness of epidermis which indicates that Nano-Lipid Carriers of MT within Nano-Lipid Carriers have better anti-psoriatic activity but MT-BL TRs a dual drug delivery gel showed similar histopathological characteristics as compared to the normal epidermis of mice, the epidermis of the skin was almost normalized.

By increased level of cytokines, psoriatic skin is characterized. TNF-α and IL-17(Tamayo, Gamazo, de Souza Reboucas, & Irache, 2017).ELISA assay was performed to find the level of TNF-α and IL-17. The increased level of pro-inflammatory cytokines IL-17, IL-23, TNF-α and IL-27 due to the activation of Th1 and Th 17 cells (CD4+T cells and CD8+T cells) enhances the inflammatory response.

The stable MT-TRs based transfersomal gel with advanced efficiency against psoriasis model in BALB/c mice was investigated for the liposomal targeted drug delivery of MT-BL TRs. The results establish that MT-BL TRs were more potent and have better penetration and permeation than single loaded drugs.

Conclusion:
It can be concluded that newer targeting strategies NLCs of dual drug delivery of Nano Lipid Carriers that could be administered topically for the treatment of Psoriasis. Furthermore, this approach opens newer avenues for continued and sustained research in pharmaceuticals with much more effective outcomes.
Limitations: In this study the sample size was small. To study more effectively ELISA and more Cytokines Assay should be applied, More Variables of surfactants and Edge Activators should be studied.
Future Perspective:
This study will create new opportunities for the release profile of topical Dual Delivery of anti-psoriasis drugs. Further evaluation on Synergistic Mechanism and cytotoxicity studies on novel co-loaded Nano-lipid carriers for the treatment of Psoriasis.
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