Fabrication and Characterization of Receptor Mediated Nanocarrier(s) for the Treatment of Inflammatory Arthritis

SUMMARY OF THE THESIS

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Summary

Inflammation has long been thought to be the body's defensive mechanism in reaction to infection or damage. The process of inflammation is predominantly a protective response mechanism generated by the immune system of the host organism as a reaction to harmful or poisonous agents, such as microorganisms. Inflammation plays a critical role in the immune defence mechanism. However, when inflammation becomes disproportionate or persistent, it can pose a significant risk to tissue integrity, resulting in tissue destruction and ultimately leading to various disease conditions. Moreover, it is becoming well-recognized that persistent inflammation is invariably related to illnesses that are associated with poor quality of life, including rheumatoid arthritis, obesity, cardiovascular diseases, neurological disorders, and cancer.

Rheumatoid arthritis (RA) is an autoimmune condition of chronic nature that mostly impacts minor joints and then gradually progresses to the main joints. Further, it may propagate to the eyes, skin, heart, kidneys, and lungs. Principally the cartilage and bone of joints are damaged, along with the weakening of tendons and ligaments. Presently the therapy comprises treatment through anti-inflammatory medications to treat symptoms from aches and inflammation, long term joint attritions are managed through glucocorticoids. Further, non-steroidal and steroidal drugs, immunosuppressive drugs, and DMARDs (disease-modifying anti-rheumatic drugs) are employed as preventive therapy for RA, and biologicals are employed for selective inhibition of immune-responsive molecules. However, conventional therapy is associated with toxicity as a fallout of long-term therapy and high doses.

The conventional method of drug delivery results in the dispersion of medicinal compounds to both targeted and non-targeted sites throughout the human body. The delivery of drugs to non-targeted sites presents certain concerns, such as an increased drug payload that may result in unintended adverse consequences. Prolonged use of such medicines is correlated with drug resistance and incremental higher dosage to achieve the intended therapeutic outcomes. This augmented drug payload can result in adverse effects on vital human organs. The emergence of such issues has prompted the necessity for the development of novel therapeutics and dosage regimens, alongside sophisticated drug delivery systems that can facilitate accurate and efficacious treatments.

The present study endeavors to devise and delineate nano-carrier mechanisms to administer drugs in a targeted manner with regulated precision, as a means of addressing the aforementioned concerns. Given the current state of research, the compounds berberine and apremilast were selected as therapeutic agents and administered via innovative lipidic nano-carriers. The nano-carriers underwent a process of design, optimization, and evaluation to assess their efficacy in drug loading and delivery. Additionally, the nano-carrier system(s) was examined for its potential to improve the management of RA.

Berberine (Br) and apremilast (Apr) have been identified as drugs with potent anti-inflammatory and immunomodulatory effects, as well as potential anti-oxidant properties. The potential of these substances to mitigate rheumatic inflammation through their anti-inflammatory and immune-modulating properties was expounded upon in sections 1.4 and 1.5 of Chapter 1, respectively. Berberine has been found to effectively inhibit inflammatory responses associated with autoimmunity *in vivo*, including RA, T1DM, EAE, and UC. This anti-inflammatory property of berberine has been demonstrated in various clinical settings. In the context of RA, berberine has demonstrated efficacy in treating CFA and other RA animal models *in vivo* through various mechanisms. These mechanisms include inducing dendritic cell apoptosis, interfering with MAPK signalling by inhibiting p-ERK, p-38, and p-JNK, attenuating Th17 activity by inducing cortistatin in the gut, restoring the equilibrium across Treg/Th17 cells, suppressing Th17 proliferation and differentiation by inhibiting CD169 and the RORγt transcription factor, inducing differentiation of Treg through aryl hydrocarbon receptor activation, and promoting anti-inflammatory M2 macrophage polarization by upregulating p-AMPK and inhibiting HIF-1α (Dinesh & Rasool, 2018; Hu et al., 2011; H. Wang et al., 2019; X. Wang et al., 2017; Z. Wang et al., 2014; Yue et al., 2017). Apremilast is a novel PDE4 inhibitor, that breakdowns cyclic adenosine monophosphate (cAMP). The cells of inflammatory nature possess PDE4, an enzyme accountable for the generation of inflammatory mediators, leading to a rise in cAMP levels, further downregulating the expression of the pro-inflammatory factor and also upregulation of interleukin 10, the anti-inflammatory factor. *In vitro* studies exhibited that apremilast effectively suppresses the generation of T-cell-derived cytokines in human T lymphocytes. (P. H. Schafer et al., 2010). Due to their lesser oral bioavailability, poor aqueous solubility, limited permeability, rapid first-pass metabolism, and instability, possible curative uses are severely constrained for both berberine and apremilast. Therefore, the incorporation of these substances (berberine and

apremilast) into a nano-particulate system respectively, was deemed significant to optimize their therapeutic efficacy. The drug compounds were obtained and verified for their purity using high-end techniques, such as NMR, FT-IR, and UV spectroscopy. The drug compounds were found to be consistent with the specifications provided by the respective manufacturers.

The UV and HPLC methods were utilized to determine the calibration curve of berberine and apremilast, respectively. In UV analysis of the drug, berberine, calibration curve showed linearity in the concentration ranges of 2-10 µg/mL in DMSO with an R^2 value of 0.9963. While via HPLC analysis, the intense peak of apremilast was observed at 3.626 and the calibration curve showed linearity in the concentration ranges of 5-30 µg/mL in acetonitrile and water (20:80) with an R^2 value of 0.9984.

The application of a targeted delivery method has the potential to transport biologically active substances to areas of inflammation with a higher degree of specificity, resulting in an increase in efficacy and a reduction in required drug dosage. Glyceryl distearate, capric acid, and oleic acid were employed as solid and liquid lipids, while triethanolamine and phospholipon 90G were used as surfactants to prepare berberine NLCs through melt-emulsification and sonication, whereas apremilast loaded Concanavalin-A modified lipidic nanocapsules were prepared, which enhance the therapeutic efficacy of both berberine and apremilast respectively, in managing rheumatoid arthritis conditions.

The melt-emulsification process, followed by sonication was used to develop the Br-loaded NLCs. A homogeneous lipid phase was produced by melting the solid (capric acid and glyceryl distearate) and liquid (oleic acid) components of the Br (10 mg) lipid phase at 55±5°C. The aqueous phase consisted of distilled water, triethanolamine (20 mg), and phospholipon 90G, which was kept at 55±5°C. The lipid phase was then mixed with the aqueous phase to produce a coarse primary emulsion and further was followed by sonication for 10 to 20 minutes (6 cycles with amplitude-80%) to obtain a nano emulsion. The NLCs dispersion was then quickly congealed over a water bath containing ice and stored at 4°C for further usage.

The optimized Br NLC mean diameter was determined to be 180.2±0.31nm (Table 4.2), with an excellent polydispersity index of 0.097±0.001, demonstrating that the formulations were homogeneous in mean diameter. The formulations in the current investigation were stable having a zeta potential value of -32.46±0.09mV. SEM was employed to observe the surface

of the NLCs. It exhibited NLCs with smooth, somewhat spherical surfaces. The entrapment efficiency of the NLCs batches ranged from 78.03 to 94.54% and for the optimized batch entrapment efficiency was found to be 88.32±2.43% while the drug loading ranged from 3.13-5.26% and for the optimized batch, it was 4.52±1.23%.

The NLCs formulation displayed prolonged drug release when the cumulative release was contrasted to the pure drug. The initial fast release of the pure drug was followed by a maximal release of up to 60-65% in 4h. But the Br NLC formulation demonstrated about 40-45% drug release in 4h and 89.33±6.65% drug release over 24 hours. The drug release for all formulations made by DoE was between 81.03±1.47 - 89.15±1.24 in 24h (Table 4.1). The NLCs formulation released 50% of the drug in 12h, with a maximum release of 61.26 ± 4.69% at the end of 24h, according to the dissolution of the NLCs in gastrointestinal pH (acidic buffer pH 1.2) (Figure 4.6). Following the 12-hour mark, the drug release remained nearly steady until the study's end. Additionally, in comparison to the release profile at pH 7.4, no discernible difference in the drug release profile was seen. At pH 1.2, there was no burst release, which is another sign that the NLCs formulation is stable at the pH of the stomach. The results indicated a significantly ($P < 0.05$) higher drug release in Br NLC at 24-hour time points respectively as compared to naïve drug. This could be due to the incorporation of the lipid layer which sustain the release of the drug showing a modified/sustain release pattern. The Higuchi model provided the greatest value of the squared correlation coefficient ($R^2 = 0.979$), which showed that this mathematical model was most suited for explaining the release of the Br from NLCs. These findings imply that diffusion regulates the release of Br from NLCs

At concentrations of 0.5 mg/mL to 2 mg/mL, negatively charged Br NLCs were found to be hemocompatible. The viability of HEK 293T cells was assessed through the MTT test after being exposed to Br and Br NLC for the incubation times of 24 and 48 hours, as shown in Fig. 5A, B, and C. After a 24-hour incubation period, the MTT assay showed that Br NLC had cell viability that was 96.54±1.65% higher than Br (91.29 ± 1.66%) at the maximum concentration of 100µg/ml (Figure 4.8). As the duration was extended to 48 h, the cell viability of Br NLC reduced as the dose of Br increased. But the viability of Br NLC was greater than 89.55±2.74% than that of Br (78.05 ± 1.83%) at the maximum dose of 100µg/ml (Figure 4.8). On the other hand, the Br NLC (6.25 µg/ml-100 µg/ml) maintained good cell viability (about 90%) at contact durations of 24 h and 48 h and demonstrated nontoxicity of NLCs at elevated concentrations. Cellular uptake of Rhodamine B labeled Br NLC by HEK

293T cells was determined through confocal laser scanning microscopy which shows intracellular uptake of the formulation.

In brief, the findings suggest that incorporating Br into NLCs has promising implications for drug delivery in the gastrointestinal tract and exhibits no notable toxicity in HEK293T cells. The results of the in vitro release studies indicated that the lipid matrix formulation effectively facilitated a controlled release of the drug. Additionally, the stability studies revealed that the NLCs remained stable over an extended storage period.

Further, when compared to control rats, the body weight of rats exposed to CFA was significantly lower. When compared to CFA induced group CFA+Br NLC-treated group displayed a significant rise in rat weight. The CFA+ Br group also showed an increase in weight but at a lower rate than that of the CFA+Br NLC treated group.

Measurement of ankle diameter at different time intervals was done. Treatment group i.e. CFA+Br NLC and CFA+ Br proved a time-dependent reduction in ankle diameter induced by CFA. On day 20, CFA-induced arthritic rats were exposed significant increase in ankle diameter (10.11±0.22mm), whereas CFA+Br NLC (6.53±0.34mm), CFA+ Br (8.33±0.17mm), and CFA+Blank NLC (10.11±0.16mm).

When compared to control rats, the organ indices (thymus and spleen) were considerably higher in CFA-induced animals. When compared to the control group CFA+Br NLC and CFA+ Br treated group exhibited a substantial decrease in organ indices. When equated to CFA induced group significant difference was found in CFA+Br NLC and CFA+ Br groups ($p<0.05$).

CFA-induced rats given Br NLC and Br had lower arthritic scores than CFA-induced rats. Rats with arthritis had a significantly higher arthritic index score than control rats. When compared to the Br NLC-treated group, Br-treated rats exhibited a similar decline in an arthritic score, but at a lower level. The hot plate test was employed to assess the pain parameters (thermal hyperalgesia). The mean baseline response time (14.00 ± 2.21 s) was not statistically changed from the control group's (14.16 ± 2.31 s) before administering CFA. After 7 days, the CFA-injected arthritic rats showed a substantial reduction in reaction time (8.33s ± 1.5) which continued to decline (3.83s ± 1.16) with a significant difference of ($P <0.05$) till the 28th day (Fig.). Curing of RA with Br NLC showed significant reversal ($P<0.05$) in thermal hyperalgesia from day 7 to day 28 (8.66s ± 2.58) to (12.20s ± 1.92) in CFA-induced arthritic rats but treatment with free Br showed less effect on rats with arthritis caused by CFA.

The study of changes in locomotion, as well as functional behavior, is done using the technique of "walking track analysis". Walking deficits were witnessed in CFA-induced rats but were improved in CFA+Br NLC and Br-treated animals with significantly lesser foot rotation and stance width ($P<0.05$). An increase in stride length was observed when compared to CFA-induced rats ($P<0.05$).

The X-ray radiographic imaging technique is a crucial tool used for analyzing the space of joint and bone degeneration caused by arthritic diseases. Radiographs of the joints for all the rat groups were obtained on the day of sacrifice, and it was seen that the rats in the CFA group had significantly greater levels of bone loss, joint degradation, and soft tissue swelling than the rats in the control group. The Br NLC-treated arthritic rats showed significantly ($p<0.05$) less erosive bone deterioration and swelling (score 1.83±0.75) and Pure Br treated animals (score 3±0.63) in contrast to the CFA-induced arthritic group.

Hepatic measures including AST, ALT, and ALP levels were not changed in normal rats, while rats that had been given CFA exhibited elevated levels of each, respectively. The ALT, AST, and ALP levels were dramatically ($P < 0.05$) decreased by Br NLC, and practically identical results were also seen in the rats in the Br group, but at a slower rate in comparison to Br NLC.

In the case of RA abnormal metabolism of glycine is well recognized as a pathophysiological feature, and the circulatory levels of glycine are mostly described to be elevated in RA. Also, circulatory levels of histidine were found to be decreased in most of the studies in the case of RA Patients. Free serum histidine levels in low amounts were also related to disease activity in RA. Some studies have reported that free histidine was found to be at a higher level in the synovial fluid (SF) as compared to the serum samples of RA (considered to be an inflammatory arthritis condition) but the level of histidine in SF was significantly less than the patients with osteoarthritis (OA, considered to a non-inflammatory arthritis condition). Therefore, the circulatory glycine to histidine ratio (GHR) was estimated and compared between the RA and NC rats. As expected in line with previous reports, the circulatory GHR levels were significantly elevated (with p-value <0.05) in the sera of RA rats (N=10; 6.416 ± 1.622) compared to the NC group of rats (N=10, 4.005 ± 1.179). Further, the circulatory GHR levels were found to be improved progressively (i.e., decreased) in the case of Br (pure drug) and Br NLC (formulation) as inferred from their respective GHR levels of 5.668±2.015 and 5.449±1.472.

ESR and WBC levels fall with an arthritic disease, whereas Hb and RBC levels rise. In the group of rats exposed to CFA, a similar outcome was seen, and Br NLC changed the hematological parameters somewhat near to the normal control**.** CFA-induced arthritis rats administered with pure Br had similar results as that of the Br NLC group but at a lower level. The values of all parameters in the arthritic and normal rats differed significantly ($P<0.05$). The arthritic-induced group had considerably higher WBC and ESR levels when compared to the control group. In contrast, a significant decrease in RBC and Hb in the arthritis-induced group was observed. Similar outcomes have been described in the literature, supporting our findings(Zhang, Liu et al. 2020). Treatment of the arthritis-induced group with the pure Br and Br NLC showed increased RBC and Hb levels while decreasing WBC and ESR levels in normal rats.

Further, the surface decoration of Br-NLCs was completed by employing the Layer by Layer (LBL) approach. Both NLCs and dextrose are negatively charged so a direct coating of dextrose over the NLCs surface is not possible owing to opposite charge repulsion. Thus, for coating an in-between intermediate layer of positively charged polymer is required which can bind to both dextrose and NLCs. For LBL coating Br-NLCs were added into chitosan solution (0.01% w/v) in a dropwise manner (3:1), with constant stirring (Tarsons-Spinot MC 01, Delhi, India) to acquire Chitosan (CH) layered NLCs (Br-CH-NLCs). The change in surface charge (zeta potential) was then measured confirming the coating as indicated by charge reversal. After charge reversal, the chitosan-coated NLCs were added to the dextrose solution (0.01% w/v) in a 3:1 ratio to obtain Br-loaded dextrose (DEX) decorated chitosan NLCs (DEX-Br-NLCs), and the charge reversal was confirmed through Zeta potential and invitro parameters were performed.

Lipidic nanocapsules loaded with Apremilast were prepared through the utilisation of the solvent-displacement technique. Before emulsification, the aqueous phase was subjected to dispersion of the hydrophilic surfactant Pluronic F68 at a concentration of 2%w/v for a brief duration. Concurrently, a distinct organic phase was generated using ethanol (0.5 ml) and acetone (9.5 ml) containing evening primrose oil (0.02%v/v), phosphatidylcholine (15.6%w/v), stearic acid (7.6%w/v), and drug (3%w/v). The organic phase was rapidly introduced into the aqueous phase containing Pluronic F68 via a syringe, resulting in the formation of a nanoemulsion, as evidenced by the solution turning milky. Subsequently, the organic solvents, namely ethanol and acetone, underwent evaporation under a vacuum with a pressure of 30 mBar and a temperature of 30 °C. To eliminate the surplus medication, the

Apr-LNCs were subjected to centrifugation at a velocity of 10,000 revolutions per minute for a duration of 30 minutes. The Apr-LNCs were meticulously gathered and subsequently rinsed with double-distilled water (DDW). The produced samples were subjected to lyophilization to obtain desiccated LNCs that could be utilised for subsequent purposes.

The surface decoration of Apr-LNCs was completed employing Lbl approach. Both LNCs and conA are negatively charged so a direct coating of con A over the LNCs surface is not possible owing to opposite charge repulsion. Thus, for coating an in-between intermediate layer of positively charged polymer is required which can bind to both conA and LNCs. For Lbl coating Apr-LNCs were added into chitosan solution (0.02% w/v) in a dropwise manner (2:1), with constant stirring to acquire Ch layered LNCs (Apr-Ch-LNCs). The change in surface charge was then measured confirming the coating as indicated by charge reversal. After charge reversal, the chitosan-coated LNCs were added to conA solution (0.02% w/v) in a 3:1 ratio to obtain Apr loaded conA decorated chitosan LNCs (conA-Apr-LNCs), and the charge reversal was confirmed through Zeta potential.

The particle size and zeta potential of conA-Apr-LNCs were measured in triplicate through a size analyser. The results showed that the particle size grows when the phospholipon 90G ratio increases, and that the particle size reduces when the concentration of surfactant increases. Apr-LNCs were discovered to have a zeta potential of −26.77±0.53 mV. The change in charge of the Apr-Ch-LNCs, which occurred as a result of chitosan coating, from −26.77±0.53 mV to +27.53±0.68 mV, served as confirmation that the modification had occurred. At the final step, conA decoration causes a reversal of charge to the negative from +27.53±0.68 mV to −27.11±0.15 mV due to its cationic nature. Although it is frequently believed that particles must have a zeta potential of less than ±20 mV to be stable, stable LNCs with significantly lower zeta potential have also been observed. In the current work, conA-Apr-LNCs with surface decorations were fabricated and afterward tested for surface morphology.

The investigation of the surface structure was conducted using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The micrographs exhibited the sphero-cylindrical morphology of the nanoparticle. The structure of the lyophilized conA-Apr-LNCs was analysed using SEM examination, while TEM analysis was employed to observe the photomicrographs of the liquid formulation. The descriptions indicate that the generated LNCs possess a uniform structure and exhibit a surface that is seemingly free of any fractures.

By assessing the DL potential and EE in Apr-LNCs, the formulations were optimized. Apr-LNCs, Apr-Ch-LNCs, and conA-Apr-LNCs were reported to have entrapment and loading efficiencies of 74.56±1.63% and 4.61±0.12%, 71.5±2.02% and 3.1±0.16%, and 68.22 ±1.31% and 3.56±0.16%, respectively. The fabrication stages that result in drug loss during preparation can be responsible for the progressive decline in entrapment efficiency.

The 24 h release study started with 2 h in simulated gastric fluid (SGF) with a pH of 1.2 and later in simulated intestinal fluid (SIF) with a pH of 7.4. Our findings imply that the proposed surface-decorated LNCs are suitable as peroral delivery vehicles and can protect Apr against potential acid degradation and also promote its release in the colon. During the first 2 h study, Apr-LNCs, Apr-Ch-LNCs, conA-Apr-LNCs, and Apr were released at rates of 29.66%, 21.66%, 16.80%, and 3.57% respectively. conA-Apr-LNCs showed a prolonged and slower biphasic release profile at pH 7.4 compared to Apr-CH-LNCs. In comparison to Apr-LNCs, where 78.43% of the cumulative release of Apr was observed in 24 h, conA-Apr-LNCs released around 59.33% of it. When compared to conA modified Apr, the release from Apr-LNCs was higher. The conA ornamented Ch coating done to the LNCs surface caused a rise in particle size could be the cause of the sustained release seen in the case of conA-Apr-LNCs whereas Apr showed early release of 95.77% within an initial 8 h with the release at pH 7.4 while at pH 1.2 very less release was obtained. Due to the Ch surface modification on the Apr-Ch-LNCs and conA-Apr-LNCs, their release profiles are identical. It should be emphasized that the targeted moiety's presence would not dramatically alter in vitro release. . Further, the obtained data underwent release kinetic models to explore the possible mechanism responsible for the Apr release from conA-Apr-LNCs. The Higuchi model provided the greatest value of the squared correlation coefficient ($R^2 = 0.983$), which showed that this mathematical model was most suited for explaining the release of the Apr from LNCs. These findings imply that diffusion regulates the release of Apr from LNCs.

The quantity of Con-A that was bound to the Apr-containing LNCs coated with chitosan was measured. The quantification of lectin was performed using the Folin-Ciocalteu reagent. The conjugation efficiency was found to be 82.07±1.07%. Additionally, it was observed that the hemolytic effect of Apr was contingent upon the dosage administered, whereby a higher dose resulted in a more pronounced hemolytic response. The experimental approach involved utilising Triton X to represent the entirety of hemolysis, while PBS was employed as a negative control. Following a 6-hour incubation period, it was observed that Apr-LNCs and

conA-Apr-LNCs exhibited a negligible level of hemolytic activity when compared to unbound drugs across a range of doses (0.5-2.0 mg/ml). The hemolytic results indicated a statistically significant decrease ($p<0.05$) in hemolysis when associated with pure Apr at all administered doses. The plausible explanation for this phenomenon is attributed to the utilisation of biodegradable and biocompatible excipients, along with natural lipids, in the formulation of the nanocapsules. Cell toxicity assays were performed on HEK-293T cell lines utilising formulations that were suitably diluted and loaded with drugs at varying concentrations of 6.25, 12.50, 25.0, 50.0, and 100 µg/ml. The viability rates of drug-loaded LNCs and conA modified LNCs were found to be non-toxic and suitable, with values of 93.26±2.24% and 95.88±2.17%, respectively, in contrast to blank LNCs which exhibited a viability rate of 98.99±0.70% at a concentration of 100 µg/ml. The results indicate that the drug-loaded LNCs were non-toxic and exhibited high biocompatibility with cells, as evidenced by cell viability of nearly 90% for all test samples up to a concentration of 100 µg/ml after 24 hours of incubation. However, after 48 hours, cell viabilities decreased with increasing concentration, with values of 76.3±3.34%, 86.37±1.37%, and 89.93±3.54% observed for Apr, Apr-LNC, and conA-Apr-LNCs, respectively, compared to blank LNCs with viability of 98.99±0.70% at a concentration of 100 µg/ml.

Concerning the cellular uptake of LNCs, HEK-293T cells were subjected to incubation with modified and unmodified formulations, both of which were labelled with Rhodamine B dye. The distribution of LNCs within the cells was subsequently examined using confocal fluorescence microscopy (CFM). The utilisation of confocal imaging revealed that the lymph node cells (LNCs) were internalised intracellularly by the human embryonic kidney 293T cells (HEK 293T cells). The analysis of the red channel indicated a predominant co-localization of the cytoplasmic region of the cell and the nano emulsion formulation. The fluorescence signals of conA-Apr-LNCs exhibited enhancement in comparison to Apr-LNCs.

The everted rat gut sac technique was employed to investigate the role of LNCs in the process of Apr intestinal absorption. The rate of transportation of Apr within the conA-Apr-LNCs was observed to be significantly higher in comparison to that of Apr-LNCs. The assessment of Apr-loaded LNCs' ability to penetrate the everted gut and circumvent the P-gp pump demonstrated a noteworthy enhancement in drug permeation, with a maximum of 87% achieved. The enhancement of intestinal permeation of Apr was observed to be approximately three-fold following the modification of LNCs. This discovery additionally

exhibited that P-glycoprotein pumps exhibit incapability to reach Apr in the altered LNCs, thereby facilitating its translocation across the intestinal barrier. The substantial enhancement in intestinal permeation can be attributed to the impact of surface functionalization of LNCs on the opening of tight junctions and the promotion of paracellular transport of Apr.

The present study employed the CFA-induced model of rheumatic inflammation and cartilage damage to assess the clinical characteristics of Apr, Apr-LNC, and conA-Apr-LNCs in their potential to ameliorate the impact of RA. Following a period of 24 hours, the sensitization of CFA initiated a physiological response in which the hind paw exhibited erythema and edoema. On the fifth day, the swelling reached its maximum level. The paws of both the CFA rats and blank-LNCs groups exhibited signs of arthritis. The administration of Apr, Apr-LNC, and conA-Apr-LNCs commenced on the fifth day of the CFA induction. Between days 15 and 30, a significant reduction in the clinical score was noted in the CFA group, accompanied by a decrease in oedema, indicating an exacerbation of arthritis. In contrast to the CFA group, the administration of blank LNCs did not elicit any significant impact on the arthritic score or paw oedema.

Radiological imaging revealed various pathological alterations in the soft tissue and bone erosion, which are considered as a characteristic manifestation of arthritis induced by CFA. The efficacy of Apr in mitigating the effects of RA has been confirmed. Furthermore, it has been reported that evening primrose oil can reduce bone resorption and mitigate inflammation. Furthermore, a radiographic evaluation of the ankle joints of the right hind paws indicated that the application of surface decoration of LNCs with conA resulted in a synergistic protective effect on bone regeneration, joint erosion, and soft tissue swelling, thereby demonstrating the efficacy of its targeted approach. In comparison to the healthy rats, the arthritic rats exhibited a noteworthy augmentation in soft tissue oedema and bone degradation in the tibiotarsal joint. The animals that received Apr-LNCs and conA-Apr-LNCs exhibited a decrease in bone degradation compared to the arthritic control group.

The arthritic group and blank LNCs formulation group exhibited a noteworthy level of expression of TNF-α and IL-6 in comparison to the normal control group, indicating the severity of arthritic inflammation. The administration of Apr-LNCs and conA-Apr-LNCs through the oral route resulted in a significant improvement in the levels of TNF-α and IL-6 expression in the serum. The Apr-LNCs and conA-Apr-LNCs exhibited higher effectiveness

compared to the Apr, which is noteworthy. In comparison to the blank LNCs and Apr-treated animals, the conA-Apr-LNCs group exhibited a noteworthy decrease in serum levels of TNF-α and IL-6.

Elevated erythrocyte sedimentation rate (ESR) and white blood cell (WBC) count are commonly observed in individuals with arthritic disease, while haemoglobin (Hb) and red blood cell (RBC) count tend to be decreased. The group of rats induced with CFA exhibited a comparable outcome, as evidenced by the alteration of haematological parameters by Apr-LNCs and conA-Apr-LNCs, which was similar to that observed in the control group. The present study investigates alterations in hepatic parameters consequent to the administration of Apr-LNCs and conA-Apr-LNCs. The hepatic parameters, namely AST, ALT, and ALP, were evaluated in normal rats and compared with those induced with CFA. The results indicated a significant increase in all three parameters in the CFA-induced animals. Apr-LNCs and conA-Apr-LNCs significantly suppressed the levels of AST, ALT, and ALP.

The levels of CRP, RF, and ADA in the serum of rats affected by arthritis were significantly higher compared to those in the control group of normal rats. The application of formulations resulted in a significant reduction of serum levels of RF, CRP, and ADA activity. The Apr-LNCs and conA-Apr-LNCs exhibited comparable outcomes to the control group that was not subjected to any experimental treatment.

The pain threshold was evaluated using a hot plate apparatus by means of the thermal hyperalgesia technique. The mean baseline response time did not exhibit any significant statistical alterations in comparison to the control group's prior to the administration of CFA. Following a period of seven days, arthritic rats that were injected with CFA exhibited a significant decrease in their reaction time, which continued to decrease with a notable disparity until the 28th day. The administration of Apr-LNCs resulted in a noteworthy amelioration of thermal hyperalgesia in CFA-induced arthritic rats, as evidenced by a significant reversal from day 7 to day 28. Similarly, the use of conA-Apr-LNCs also led to a significant reversal of thermal hyperalgesia in the aforementioned rats during the same time period. Conversely, the efficacy of free Apr in treating CFA-induced arthritis in rats was comparatively lower.

The *in-vivo* pharmacokinetic effects of Apr, Apr-LNCs, and conA-Apr-LNCs following oral delivery were measured. A non-compartmental design was used. The peak serum concentration (Cmax) of Apr was 20.73 µg/mL, and it took 2 h for it to reach its

maximum concentration (Tmax), which was lower than that of Apr-LNCs, and conA-Apr-LNCs. The pharmacokinetic profile of conA-Apr-LNCs (Cmax = 89.26 µg/mL) was higher than that of Apr-LNCs (Cmax = 80.35 µg/ml). Plasma concentrations for Apr, Apr-LNCs, and conA-Apr-LNCs reached 50% ($t_{1/2}$) at 6.85, 11.36, and 13.62 h, respectively. For Apr, Apr-LNCs, and conA-Apr-LNCs, the total area under the curve (AUC) was 183.66, 1677.60, and 1862.360 µg h/ml, respectively. The AUMC for Apr, Apr-LNCs, and conA-Apr-LNCs shows that conA-Apr-LNCs have the greatest AUMC, followed by Apr-LNCs and Apr. The AUMC is also employed in the MRT calculation, which reveals that conA-Apr-LNCs have the maximum residence duration, followed by Apr-LNCs and Apr. The elevated readings for conA-Apr-LNCs and Apr-LNCs indicate that it is a controlled-release formulation, which extends the drug's residence time in the body and improves the bioavailability of Apr, which can result in further dosage reduction. Decreased clearance rates and an increase in distribution volume are other indicators of the system's effectiveness.

The study evaluated the stability of conA-Apr-LNCs over a period of six months at specific intervals under varying temperature conditions, including ambient temperature (25±5 ºC), refrigeration (4±1 ºC), and 40 ºC±2ºC. At predetermined intervals, the samples were extracted and analysed to assess their stability based on particle size and medication loading as predictive factors. The findings of our inquiry revealed that the storage stability of the formulations we developed remained unaffected by particle size and DL for a duration of six months, across all three conditions. Hence, it can be inferred that the formulations exhibited stability for a minimum of six months.

The results of both formulations demonstrated effective administration of the chosen medication in a precise manner for the treatment of rheumatoid arthritis. To facilitate the release of berberine and apremilast, the lipid-based nanocarrier employed a matrix system to achieve regulated delivery of berberine and apremilast. The study revealed that the lipidic nanoparticles exhibited cytocompatibility and hemocompatibility, and facilitated targeted drug delivery for anti-rheumatic purposes.

The hemo and cyto-compatible nano-carriers were developed for the purpose of delivering the bioactive ingredient berberine and the chemical constituent apremilast, both of which were tested for their ability to reduce inflammation. The application of nanostructured lipid carriers, specifically lipidic formulations, presents a viable option for the transdermal administration of berberine and apremilast. This approach serves to bypass hepatic

degradation, ultimately enhancing the anti-inflammatory and antioxidant activities of these compounds within the joint synovium. The intended outcome is the mitigation of rheumatic inflammation and the alleviation of associated symptoms of rheumatoid arthritis. Both formulations exhibited enhanced pharmacokinetic properties and demonstrated promising potential as effective candidates for the controlled release of the aforementioned drugs. However, additional clinical trials are required to confirm the efficacy of the formulations in human delivery.

Nanotechnology is poised to undergo a significant breakthrough in virtually all fields of science and technology. The aspiration for achieving high end techniques is currently within our reach, however, the initial step towards realising this goal involves the development of a stable and effective nanocarrier system. This system possesses the capability to targeted delivery of the drugs at intended sites.

The Br NLCs were prepared successfully. The findings of the investigation demonstrate that the incorporation of Br into NLCs exhibits promising prospects for proficient drug delivery to the intended target location, as evidenced by the protective effect on the enclosed drug during transportation against the extensive pH fluctuations throughout the gastrointestinal tract. Furthermore, the HEK293T cells did not demonstrate any significant toxicity. The results of the in vitro release studies indicate that the lipid matrix formulation effectively facilitated controlled drug release. Furthermore, the stability studies revealed that the NLCs remained stable over an extended storage period.

Furthermore, the Concanavalin A (conA)-decorated Lipid Nanocapsules (LNCs) were efficiently formulated for the administration of Apr to CFA-Induced Rheumatoid Arthritis (CFA-induced RA). The findings indicate that the formulation of conA-Apr-LNCs can be optimised and achieved through the utilisation of different concentrations of lipid, surfactant, and pluronic F68. The results of the *in vitro* release studies indicate that the lipid matrix of the formulation facilitated the controlled release of the drug via conA-Apr-LNCs, thereby potentially enhancing the efficacy of the dosage regimen. Pharmacokinetic investigations have revealed that conA-Apr-LNCs demonstrate significantly increased bioavailability compared to the naive drugs. Simultaneously, the *in vivo* findings revealed that conA-Apr-LNCs exhibit enhanced therapeutic effectiveness in the treatment of rheumatoid arthritis, as evidenced by radiographic images, biochemical assessments, inflammatory markers, and thermal hyperalgesia. The findings of our study demonstrate that

the utilisation of conA-Apr-LNCs has resulted in a notable enhancement in the therapeutic efficacy for the management of rheumatoid arthritis in the animal model induced by CFA.

Although it is impossible to predict what the future holds for nanoscience and nanotechnology, it is undeniable that the area has a good potential for a brighter future and that it will surely have a significant impact on societal needs.