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NANOEMULGEL-BASED DELIVERY OF 3-ACETYL-11-KETO-B-BOSWELLIC ACID: A STRATEGY FOR ENHANCED TRANSDERMAL DRUG DELIVERY AND ANTI-ARTHRITIC EFFICACY

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune condition that causes inflammation of the synovial membrane, leading to joint pain, swelling and destruction. 3-O-acetyl-11-keto-β-boswellic acid (AKBA), a constituent of *Boswellia serrata*, effective against inflammation and arthritis, but suffers from limited aqueous solubility and permeability through skin. In this regard, the objective of the current study was to prepare AKBA loaded nanoemulgel and explore its therapeutic potential in rheumatoid arthritis.

Methods: An optimized AKBA nanoemulsion was added into gel matrix prepared with HPMC K15M and carbopol 940 to formulate nanoemulgels. These formulations were evaluated for physicochemical properties, *in-vitro* drug release and ex-vivo skin permeation. The optimized formulation was then tested in an arthritis animal model induced with complete freund's adjuvant (CFA). Haematological, serological parameters were estimated and radiological, histopathological examination of ankle joints was also conducted.

Results: Nanoemulgel formulation NG5 made of carbopol 940 was chosen as the optimized nanoemulgel in terms of physicochemical properties and drug release properties. NG5 had the following physical characteristics: pH of 6.11 ± 0.04, viscosity of 538.42 ± 2.84 cP and drug content of 105.12 ± 0.08%. The formulation had 93.42% drug diffusion cumulatively over 24 h. In *ex-vivo* permeation studies, cumulative permeation was found to be 1487.32 ± 8.46 µg/cm², with a steady state flux of 68.60 µg/h/cm²; flux was 2.58 times higher than that of conventional gel. In induced arthritis, AKBA nanoemulgel administration gradually decreased paw thickness and degree of arthritis. The treatment alleviated haematological abnormalities associated with the disease and decreased serum RF, CRP, TNF-α, IL-6 and IL-1β levels compared with the disease control group. Radiologically, joint architecture was conserved with lesser swelling and bone erosions of joints, while histopathologically, there was attenuation of cartilage erosion and synovitis.

Conclusion: It was clear from the above results that the AKBA nanoemulgel increased transdermal penetration and sustained release of drug with significant anti-arthritic and anti-inflammatory activity in experimental rheumatoid arthritis. Therefore, it was concluded that AKBA nanoemulgel could be a useful transdermal approach for the treatment of rheumatoid arthritis.

KEYWORDS: 3-O-Acetyl-11-keto-β-boswellic acid, nanoemulgel, transdermal drug delivery, anti-inflammatory activity and anti-arthritic activity.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and systemic autoimmune inflammatory disease with features of persistent inflammation of the synovium, cartilage destruction, bone erosion, and joint dysfunction[1]. This disease is associated with high morbidity, significantly limiting mobility and quality of life[2]. The inflammatory process involved in the pathogenesis of RA is linked to the activation of immune cells and secretion of pro-inflammatory mediators, such as TNF-α, IL-6 and IL-1β. These agents are responsible for the inflammation and destruction of the synovium, cartilage degradation, and structural joint destruction. Therefore, the treatment of this condition involves continuous inflammation inhibition and prevention of joint degradation[2].

The pharmacological therapy of RA includes NSAIDs (non-steroidal anti-inflammatory agents, glucocorticoids, and DMARDs (disease modifying anti-rheumatic drugs)[3]. While these medications demonstrate significant therapeutic effects, their long-term application might lead to a number of side effects, such as gastrointestinal, renal, and hepatic disorders, among others[4]. Besides, the oral drug delivery allows the drug to experience the influence of the processes occurring in the gastrointestinal tract and liver, leading to suboptimal systemic levels of the drug. Thus, there is a need to develop an alternative method of drug delivery providing better efficacy and reduced systemic exposure[5].

Transdermal drug delivery is a promising alternative in the administration of suitable drugs. It can allow sustained drug delivery, bypassing the first-pass effect from the gastrointestinal tract and enhancing patient compliance[6]. Nevertheless, the stratum corneum is considered the main barrier of transdermal drug delivery, especially for drugs having low solubility and poor skin penetration capacity[10]. For this reason, formulation techniques that enhance drug solubility, increase interfacial area, and promote skin permeation are needed to develop an efficient transdermal delivery system for poorly water-soluble anti-inflammatory drugs[7].

Biologically active natural products (phytochemicals) have gained much interest as drug candidates for inflammatory diseases due to their broad pharmacological effects. *Boswellia serrata* or Indian frankincense contains several pentacyclic triterpenoids called boswellic acids. 3-O-acetyl-11-keto-β-boswellic acid (AKBA) is one of these compounds which gained much attention owing to its anti-inflammatory and anti-arthritic properties[8,9]. The pharmacological action of AKBA is thought to be related to inflammation pathway modulation, such as inhibition of 5-lipoxygenase-mediated leukotrienes production and inflammation mediators of chronic inflammation[9].

However, despite its favorable pharmacological profile, AKBA is limited in terms of its pharmaceutical development because of physiochemical properties, such as low aqueous solubility and poor bioavailability. Such physicochemical properties may limit its ability to dissolve and move through biological membranes. Therefore, development of a proper delivery system which will help in enhancing its solubilization and transdermal permeability may help to improve the therapeutic value of AKBA[11]. Recent studies on the nano-based delivery and transdermal delivery of AKBA suggest the possibility of using nanocarriers for the delivery of AKBA[10]. Nevertheless, the therapeutic effectiveness of AKBA nanoemulgel must be confirmed through comprehensive evaluation of its formulation, release, permeation, and *in-vivo* anti-arthritic activity[13].

2. MATERIALS AND METHODS

3-Acetyl-11-keto-β-boswellic acid was obtained as a gift sample. Carbopol, HPMC and propylene glycol were purchased from Loba Chemie Ltd., Mumbai, India. Chemicals and reagents used in this study were analytical grade.

3. Formulation of AKBA loaded nanoemulgels

AKBA nanoemulgels was prepared by incorporating the drug loaded nanoemulsion in gel base. AKBA loaded nanoemulsions (NE) was prepared with 2% virgin coconut oil (VCO)[16] was used as the oil phase along with 8% (w/w) Smix, which contained tween 80 and propylene glycol in the ratio of 1:2. Selected ingredients were mixed properly and then formulated to AKBA-loaded nanoemulsion using 10,000 rpm speed for 30 min[14,15]. Then nanoemulsions were incorporated in gel base with two different gelling agents, HPMC K15M, and carbopol 940, in different concentrations. Four different nanoemulgels (NG1-NG4) based on HPMC K15M have been prepared by taking the concentrations of HPMC K15M at 2.5%, 3.0%, 3.5%, and 4.0% (w/w). Required quantities of HPMC K15M was slowly added to distilled water under constant stirring and left overnight at room temperature to hydrate and swell in order to get the gel base. Nanoemulsion was slowly added to the hydrated gel base in the ratio of 1:1 while continuously stirring for about 15 min to form the nanoemulgel[17].

Likewise, four different nanoemulgels (NG5-NG8) based on carbopol 940 were prepared by taking the concentrations of carbopol 940 at 0.4%, 0.6%, 0.8%, and 1.0% (w/w). Carbopol 940 was first dissolved in distilled water and hydrated to obtain the gel base. Then the pH of the gel was adjusted to 6.5-7.0 by slow addition of triethanolamine (TEA). Finally, AKBA-loaded nanoemulsion was added to the carbopol 940 gel base in the ratio of 1:1 to obtain the nanoemulgel[18,19].

For comparative analysis, two types of conventional AKBA gel preparations were formulated. G1 was made using 0.5% (w/w) AKBA dispersed in the HPMC K15M gel matrix, while G2 was made using 0.5% (w/w) AKBA dispersed in the carbopol 940 gel matrix. These conventional gels were prepared following the respective gel matrix preparation procedures without incorporating the nanoemulsion[20].

Both nanoemulgels and conventional gels were further characterized for their appearance, pH, viscosity, spreadability, drug content, in-vitro drug release, and ex-vivo skin permeation for selection of the optimized formulation for AKBA transdermal delivery[21,22].

Table 1: Formulation of AKBA nanoemulgels and gels

Ingredients (% w/w)	NE	NG1	NG2	NG3	NG4	NG5	NG6	NG7	NG8	G1	G2
AKBA	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Coconut oil	2	2	2	2	2	2	2	2	2	-	-
Smix	8	8	8	8	8	8	8	8	8	-	-
HPMC K15M	-	2.5	3	3.5	4	-	-	-	-	4	-
Carbopol 940	-	-	-	-	-	0.4	0.6	0.8	1	-	1
Glycerol	1	1	1	1	1	1	1	1	1	1	1
Water (Q.S)	100	100	100	100	100	100	100	100	100	100	100

4. Characterization and evaluation of nanoemulgel

4.1. Homogeneity

The prepared formulations of nanoemulgel were assessed for homogeneity visually after reaching equilibrium at room temperature. The formulations were assessed for their homogeneity in terms of their appearance, consistency, color, transparency, and freedom from phase separation, grittiness, air bubbles, or particulates. A homogeneous formulation that lacks visible aggregates was accepted for further studies[23].

4.2. Viscosity
Viscosity of the nanoemulgel formulations were evaluated using brookfield viscometer.

4.3. Drug content
5mg of equivalent AKBA nanoemulgel was dissolved in ethanol and then made up to 100mL with pH 7.4 phosphate buffer. The solution was stirred for 30 min and filtered through 0.45 µm filter to separate undissolved polymeric material. 10 µg/mL solution was prepared from the stock solution and absorbance was calculated by using UV-visible spectropohotometer at 255 nm. Drug content in nanoemulgel was estimated in percentage and runs were carried out in triplicate[24].

4.4. Extrudability
The extrudability of the nanoemulgel was determined by the use of tube extrusion technique to determine the ease of extruding the sample from a collapsible aluminum tube. Nanoemulgel was loaded into an aluminum tube and the tube appropriately sealed. Force was placed on the tube and the volume of the gel extruded through the nozzle for 10 sec was recorded. Alternatively, the force needed to extrude a 0.5 cm ribbon of gel was determined and results expressed in grams. Nanoemulgels that could easily be extruded without too much force were said to have good extrudability. This test was done in triplicates[25,26].

4.5. Spreadability
Slip-and-drag method was used to assess the spreadability of the nanoemulgel. The emulgel was put between two glass plates (20 × 20 cm) and one gram of it was placed. Uniform spreading was achieved by placing the 500 g on the upper plate for 5 min and the diameter of the spread was then measured on two axes at right angles to one another and the mean diameter recorded33. A weight of 50 g was fastened to the upper plate and the time taken for the plate to move a fixed distance of 10 cm was recorded for the determination of drag time. The equation used for the calculation of spreadability was $S = (M \times L)/T$ with M being the applied weight, L the distance travelled, and T the time taken. The measurements were performed three times at 25 ± 2 °C[26].

4.6. *In-vitro* studies of drug release
In-vitro drug release study was carried out in franz diffusion cell with dialysis membrane. Phosphate buffer solution (pH 7.4) was put into the receptor compartment and stirred continuously at 600 rpm at 32 ± 0.5 °C. The formulation equivalent of 5 mg Boswellic acid was placed in the donor compartment. Aliquots were removed at regular time intervals (0.5–24 h) and immediately replaced with the same volume of fresh receptor medium to keep the sink conditions27. All the samples were subjected to UV-VIS Spectrofluorometric analysis at 255 nm. The release kinetics of the drug was determined by fitting the released data to the zero-order, first-order, higuchi and korsmeyer–peppas kinetic models. The release profile of the optimized nanoemulgel was compared with that of the conventional 1% Carbopol based AKBA gel[28].

4.7. *In-vitro* skin permeation studies
In-vitro skin permeation study was performed with franz diffusion cells with semipermeable membrane of dermatomed porcine ear skin (thickness: 0.8–1.0 mm). Phosphate buffer (pH 7.4) was used as a receptor compartment, kept at 32 ± 0.5 °C and continuously stirred at 600 rpm during the experiment[28]. Formulation evenly applied on the skin surface in a diffusion area of 1 cm². Samples were taken at fixed time intervals over a 24 h period, and an equal volume of fresh receptor medium was added to replace each sample that was withdrawn, to ensure constant diffusion conditions. The samples were analyzed spectrophotometrically at 255 nm. The cumulative amount of drug permeated, the flux and the permeability coefficient (Kp) were calculated. Each experiment was repeated three times[29,30].

5. *In-vivo* anti-arthritic activity of the optimized AKBA nanoemulgel
This study was performed to assess the effect of AKBA nanoemulgel for the management of pain and synovial inflammation of joints in RA. The protocol required for the study and the number of animals required, were approved by the IAEC with protocol number, 18/IAEC/SVCP/2025. *In-vivo* anti-arthritic activity study was done using healthy adult female albino rats of wistar breed having weight 180-220 g. with 12 h light and 12 h dark period. Standard pellets diet and purified water were fed to the animals[34,35].

Study design
Group I (Control group): 0.5 mL of 0.9% w/v saline solution
Group II (RA induced Control – disease group): Complete Freund's Adjuvant (CFA) adjuvant
Group III (Standard): CFA+DFC - Diclofenac sodium gel (10 mg/kg body weight)
Group IV: CFA+NG5 – formulation NG5
Induction of experimental arthritis was done by using CFA, which is an extensively used method that closely mimics human RA in terms of chronic inflammation, synovitis, cartilage damage, and bone erosion. All groups of animals except the normal control group were injected with 100 µL CFA, containing 5 mg/mL heat killed mycobacterium tuberculosis in liquid paraffin under the plantar region of the right hind paw using a sterile 26 gauge needle. Paw edema in the animal models was confirmed 6 h post-CFA induction and this was considered as the baseline (day 1) of induction. Treatment

regimen was begun on the induction of arthritis and continued till the 28th day. Throughout the duration of the experiment, animals were monitored for changes in behavior, body weight, mobility, and clinical signs of arthritis[34].

5.1. Body weight measurement

Body weight was measured before inducing arthritis and then on the 4th, 10th, 14th, 21st, and 28th days using a digital weighing balance. Weight loss is one of the features of chronic inflammatory disorders because of high metabolic needs, reduced food consumption, and muscular atrophy. Increased body weight after therapy can serve as an indirect marker of therapy efficacy and general health condition.

5.2. Measurement of change in joint diameter

Change in joint diameter was measured using a digital vernier caliper on day 0 before CFA injections and thereafter on 4th, 10th, 14th, 21st, and 28th day. The change in joint diameter was calculated as the difference between the final and initial joint diameter[32].

5.3. Arthritis severity score measurement

The severity of the clinical disease was assessed using the blinded visual scores. Visual assessment of the severity of the disease in each paw was done. The score given depend up on the level of functional disability and inflammation present in the affected limbs[6]. Score 0 indicates no squeaking and no leg withdrawal, 1 represents either squeaking or leg withdrawal, 2 indicates both squeaking or leg withdrawal, 3 indicates walking without touching the injected paw.

5.4. Haematological analysis

On the 29th day after completing the treatment regimen, blood samples were collected through retro-orbital plexus puncture method into ethylene diamine tetra acetic acid (EDTA) coated tubes. Haematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and PCV were determined by usual standardized laboratory method[31].

5.5. Serum biochemical analysis

On the 29th day of the experiment, blood samples were collected from the retro-orbital plexus to sterile non-heparinized tubes and left to clot at room temperature for 30-45 min. Samples were centrifuged at 3,000 rpm for 10 min to isolate serum. Serum samples were kept at -20°C until further biochemical analysis. The levels of inflammatory mediators like IL-6, TNF-α, CRP, RF, IL-1β were evaluated through enzyme-linked immunosorbent assay (ELISA) kits[30].

5.6. Histopathological examination of ankle joints

On 29th day, the rats were euthanized by means of cervical dislocation technique and the ankle joints from hind limbs were carefully removed, washed with normal saline to remove blood and connective tissues, and fixed in 10% neutral buffered formalin to preserve tissue morphology properly. Decalcification of the fixed samples was done in 10% ethylenediaminetetraacetic acid (EDTA) solution for 2-3 weeks. The slices were mounted onto the glass slides and stained with hematoxylin and eosin (H&E). The degree of histopathological lesions in each experimental group was compared with those of the normal and disease control groups. Histopathological findings were then correlated with clinical signs, hematological indices, biochemical parameters, and radiological investigation to confirm the overall effectiveness of the nanoemulgel[35].

5.8. Statistical analysis

All experimental data were represented as mean ± standard error of mean (SEM) for six animals in each group. Graphpad prism version 8.0.2 was used for all statistical analyses. One-way analysis of variance (ANOVA) followed by the tukey's multiple comparisons test was used for parametric data and kruskal-wallis test was done for non-parametric arthritis scores. Significant values were taken at $p < 0.05$ level[28].

6. RESULTS AND DISCUSSION

6.1. Evaluation of the formulations of AKBA nanoemulgels

Table 2: Evaluation parameters of AKBA nanoemulgels

Formulation code	pH	Viscosity (%)	Drug content (%)	Extrudability (g/cm^2)	Spreadability (g.cm/sec)
NE	5.6±0.4	53.5±0.18	102.86± 0.14	-	-
NG1	6.10±0.02	428.52 ± 4.03	103.26± 0.24	1425±18	34.82±0.42
NG2	5.84±0.02	446.48±3.46	94.52± 0.16	1392±16	33.54±0.38
NG3	5.99±0.03	488.42±6.84	105.54± 0.28	1338±20	31.68±0.41
NG4	5.96±0.02	520.53±4.18	98.38± 0.26	1286±17	29.82±0.36
NG5	6.11±0.04	538.42±2.84	105.12± 0.08	1258±15	28.96±0.32
NG6	5.84±0.02	548.36±2.16	100.26± 0.18	1235±16	28.12±0.34
NG7	5.99±0.03	560.42±4.32	106.10± 0.28	1212±18	27.24±0.30

NG8	5.64±0.04	588.42±8.14	99.26± 0.42	1178±19	25.86±0.28
G1	5.02±0.03	492.18±6.32	99.42±0.39	1325±18	31.26±0.39
G2	5.48±0.01	560.12±5.16	98.28±0.24	1208±17	27.42±0.31

DISCUSSION

Physical appearance and homogeneity of all nanoemulgel formulations were evaluated visually. All nanoemulgel formulations were found to be smooth, homogeneous, and free from lumps, grittiness, phase separation, and air bubbles, thus showing successful incorporation of the optimized nanoemulsion into the gel matrix. There was no sign of drug crystallization and aggregation during the entire process, suggesting the homogenous presence of boswellic acid in all formulations. While the conventional gel formulations were homogeneous too, the nanoemulgels were smoother compared to conventional gels due to their nanosized dispersed nature.

The pH value of all nanoemulgel formulations ranged between 5.64 ± 0.04 to 6.11 ± 0.04, falling within the physiologically acceptable skin pH range (5.5-6.5). It can be concluded that the obtained nanoemulgels will not irritate the skin. The viscosity of the nanoemulgels was directly proportional to the polymer concentration used in them. The HPMC K15M based nanoemulgel formulations (NG1–NG4) had viscosities from 428.52 ± 4.03 to 520.53 ± 4.18 cP, whereas the carbopol 940 based nanoemulgel formulations (NG5–NG8) had comparatively high viscosities from 538.42 ± 2.84 to 588.42 ± 8.14 cP. This is due to the highly crosslinked structure and swellability of carbopol 940 polymer, which makes the gel network more viscous as compared to that made by HPMC K15M polymer. Also, the conventional carbopol gel (G2) was more viscous as compared to HPMC gel (G1).

All nanoemulgel formulations had drug content values between 94.52 ± 0.16% to 106.10 ± 0.28%, showing efficient incorporation and uniform distribution of AKBA in the gel matrix. The extrudability assessment indicated that all the nanoemulgels were easily extruded from collapsible tubes under the influence of moderate force. The HPMC K15M nanoemulgels were more extrudable as compared to the carbopol 940 nanoemulgels due to their low viscosity. On the other hand, the higher the concentration of carbopol 940 in the nanoemulgels, the more extrusion force was needed due to dense polymeric network formation. However, all formulations possessed sufficient extrudability characteristics which ensured easy application.

Spreadability of nanoemulgels was inversely proportional to viscosity. Thus, the formulation containing lower gelling agent concentrations had higher spreadability characteristics and made their application easy and uniform on the skin surface. With the increase in the concentrations of HPMC K15M and carbopol 940, spreadability characteristics of nanoemulgels reduced due to the rise in viscosity. HPMC K15M nanoemulgels had comparatively better spreadability characteristics than carbopol 940 nanoemulgels due to their low-viscous gel matrix.

6.2. *In-vitro* diffusion studies of AKBA nanoemulgels

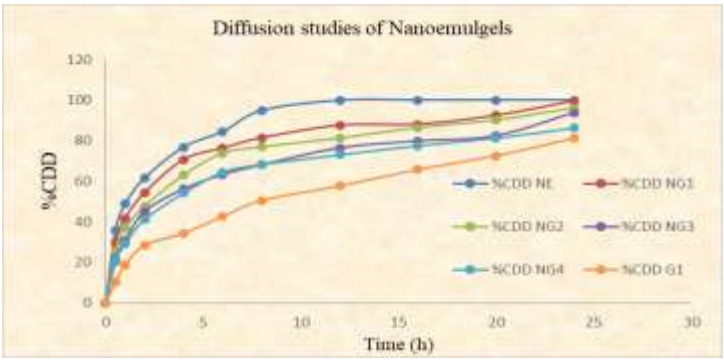


Fig. 1: *In-vitro* diffusion of AKBA nanoemulgels containing HPMC as gelling agent

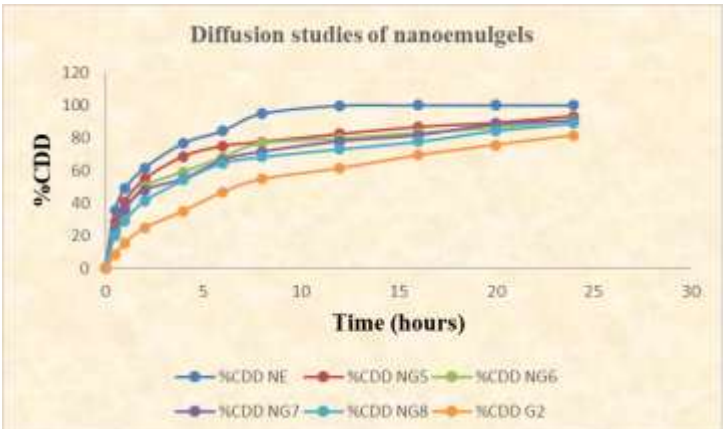


Fig. 2: *In-vitro* diffusion of AKBA nanoemulgels containing carbopol as gelling agent

Discussion: Cumulative *in-vitro* drug diffusion studies from nanoemulgels over a 24 h period revealed a steady and controlled release of the AKBA from all formulations. Nanoemulsion, exhibited rapid drug release rate i.e., 99.88% diffusion was noted at 12 h, indicating very fast drug diffusion up to near completion level. The high rate of diffusion of the drug is due to small droplet size, higher surface area, and improved solubilization of boswellic acid within the nanoemulsion system. The nanoemulgels prepared with HPMC K15M gave high drug diffusion up to 99.64% at 24 h in NG1, followed by 96.22%, 93.80% and 86.32% drug diffusion in NG2, NG3 and NG4 respectively. As the concentration of HPMC K15M increases in the formulation, the diffusional path length was increased due to increased viscosity of the gelling agent.

Nanoemulgels containing carbopol 940 also gave sustained drug diffusion with 93.42% CDD for NG5 at 24 h followed by 91.11%, 90.42% and 88.88% diffusion in NG6, NG7 and NG8 respectively. This decrease in drug diffusion with increase in concentration of carbopol is due to increased polymer cross linkage. According to the physicochemical analysis and *in-vitro* drug diffusion studies, the optimized nanoemulgel formulation was found to be NG5, which comprised of 0.4% carbopol 940. Gels possessed relatively less drug diffusion compared to their respective nanoemulgels. Formulation NG5 showed a suitable pH value (6.11), moderate viscosity (538.42 cP), good drug content (105.12%), excellent spreadability and extrudability, and sustained cumulative drug diffusion (93.42%). The moderate viscosity of the formulation due to the presence of carbopol 940 increased the residence time of the formulation in the skin while ensuring effective drug diffusion from the formulation.

In-vitro release of drugs was modeled on different mathematical models, which include zero-order, first-order, higuchi and korsmeyer-peppas to identify the drug diffusion mechanism of AKBA from the nanoemulgel formulations. In addition, the release exponent indicates the anomalous (non-Fickian) diffusion, which implies that the release mechanism was controlled by both diffusion and relaxation/erosion of hydrated polymeric matrix.

6.3. *Ex-vivo* diffusion studies of AKBA nanoemulgels

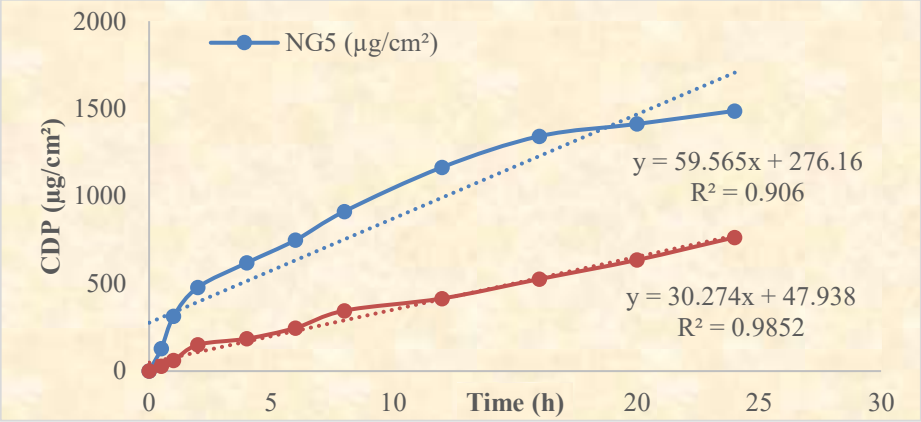


Fig. 3: *Ex-vivo* diffusion studies of AKBA nanoemulgels

Table 3: Permeation kinetics of NG5 nanoemulgel

Type of formulation	Cumulative drug permeated (µg/cm²)	Jss (µg/h/cm²)	Permeability coefficient, Kp (cm/h)	Enhancement ratio
NG5	1487.32± 8.46	68.60	0.0137	2.58
G2	764.36 ± 15.22	26.47	0.0053	-

The optimal nanoemulgel (NG5) of AKBA showed a 2.58 fold increase in flux compared to that of the normal gel (G2). This clearly shows an increased permeation of the AKBA through the skin. Additionally, the increased Kp and reduced log Kp values clearly show that the optimized nanoemulgel was effective in increasing the permeability of AKBA through the skin. The reason behind this increased permeability could be the small size of the nanoparticles, solubility of AKBA, high interfacial surface area, and the enhanced permeation property of tween 80 and propylene glycol.

7. *In-vivo* anti-arthritic activity of NG5 nanoemulgel

7.1. Measurement of body weight

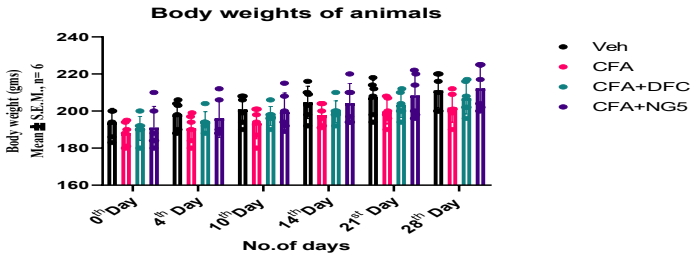
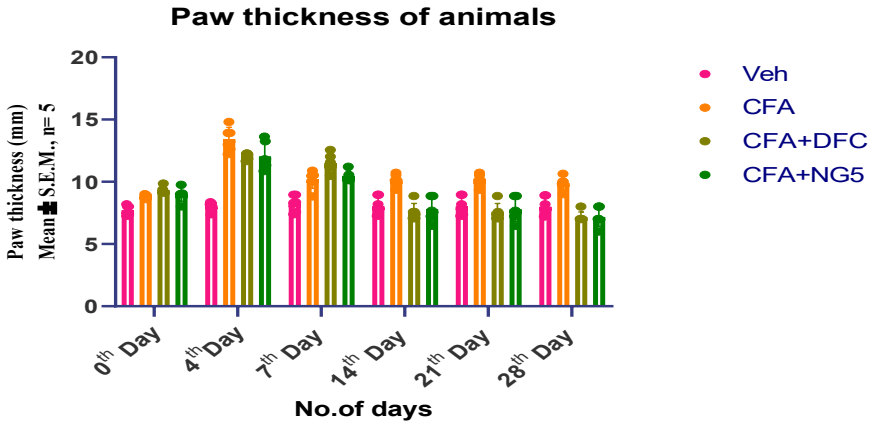


Fig. 4: Body weights of groups at definite time intervals

Body weights on 0th, 4th, 10th, 21st and 28th days were expressed as Mean±S.E.M. p values were <0.05, considered as statistically significant.

Discussion: CFA control animals showed minimum gain in body weight during the entire experiment period, reflecting the negative consequences of chronic inflammation due to rheumatoid arthritis. Conversely, CFA+DFC, and CFA+NG5 treatment caused a significant increase in body weight as compared to CFA control animals. Out of all the treatment groups, the CFA+NG5 group showed the most pronounced increase in body weight reflecting the efficacy of treatment regarding the reduction in body weight loss. The third treatment group was CFA+DFC, and the least increase in body weight was seen in the CFA control group.

7.2. Measurement of paw thickness

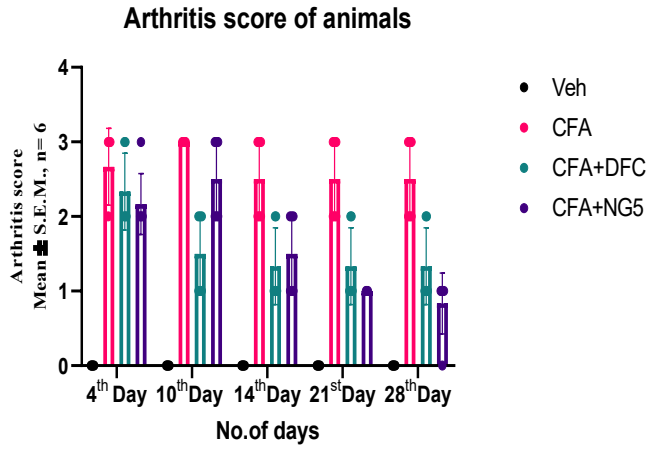


Thickness on 0th, 4th, 10th, 21st and 28th days were expressed as Mean±S.E.M. p values were <0.05, considered as statistically significant.

Fig. 5: Paw thickness of groups at definite time intervals

Discussion: CFA+DFC, CFA+NG5 showed significant decrease in paw thickness when compared to the CFA control group after day 10 of the experiment. While all the treatment groups showed progressive decrease in paw edema, the CFA+NG5 group showed the most significant decrease in paw thickness among all groups, followed by CFA+DFC group. The 28th day revealed that the combination treatment recovered paw thickness closer to the normal vehicle group.

7.3. Arthritis severity score measurement



Arthritis severity score on 0th, 4th, 10th, 21st and 28th days were expressed as Mean±S.E.M. p values were <0.05, considered as statistically significant.

Fig. 6: Arthritis severity score of groups at definite time intervals

Discussion: CFA+DFC and CFA+NG5 led to significant suppression of the arthritis score compared to the CFA control group. This suppression became obvious starting from the 10th day, showing the therapeutic value of the transdermal patches used in suppressing disease progression. In all the experimental groups of animals that received various treatments, highest reduction in arthritis score observed by the CFA+NG5 and then CFA+DFC groups. The lowest arthritis score was observed at the 28th day among the tested groups, with a score similar to the normal vehicle group, thus showing that a notable improvement had been made in the condition of the joints and in the manifestation of rheumatoid arthritis.

7.4: Haematological analysis

Table 4: Haematological parameters for different groups

Group	RBC ($X10^6$ µL)	WBC ($X10^3$ µL)	Hb (g/dL)	ESR (mm/1st hr)	PCV (%)
Control	5.46	10.42	13.50	4.2	50.46
Disease	4.38	15.65	9.40	11.8	27.3
Standard	5.08	9.28	12.56	7.2	45.58
NG5	6.24	10.40	14.68	5.3	49.48

Discussion: Changes in the haematological parameters were quite remarkable for the CFA group because there was a noticeable reduction in the number of red blood cells (4.38×10^6/µL), hemoglobin (9.40 g/dL), and packed cell volume (27.3%) and an increased white blood cells count (15.65×10^3/µL) and erythrocyte sedimentation rate in comparison to the normal control group. NG5 resulted in more considerable recovery, as hematological parameters approached those of the control group. The normalization of RBC, Hb, PCV, WBC, and ESR indicates effective suppression of systemic inflammation, improvement of anemia associated with rheumatoid arthritis, and recovery of normal physiological status.

7.5: Serum biochemical analysis

Table 5: Serum analysis for different groups

Group	RF	CRP (mg/dL)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)
Control	6.8	1.8	10.4	64.2	30.2
Disease	62.3	8.8	50.4	148.4	80.4
Standard	46.5	4.8	25.6	100.2	42.0
NG5	28.2	3.2	28.6	80.1	58.4

Discussion: From the results, it was evident that there was an elevation of inflammatory biomarkers like rheumatoid factor (RF), C-reactive protein (CRP), TNF-α, IL-6, and IL-1β due to induction of RA with CFA compared to normal control group. From the standard formulation, there was an observed significant reduction of inflammatory biomarkers compared to the disease group, thus there was a successful suppression of systemic inflammation. From the AKBA nanoemulgel (NG5), there was a more significant reduction in RF and CRP biomarker along with a decrease in pro-inflammatory cytokine levels. The significant decrease in TNF-α, IL-6, and IL-1β suggested effective inhibition of the main pro-inflammatory cytokines responsible for the inflammation of the synovial membranes, cartilage destruction, and bone damage in RA.

7.6. Histopathological examination of joints

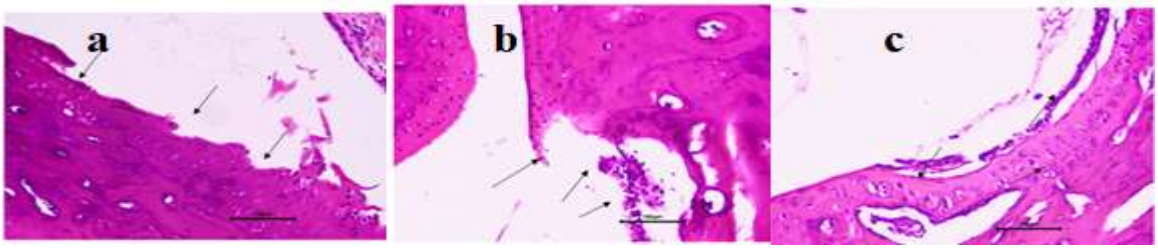


Fig. 7: Histopathology of joint a) Disease b) CFA+DFC c) CFA+NG5

DISCUSSION

Histopathological analysis of the joints' tissue sections revealed a clear distinction among the groups used for the experiment depending on the severity of arthritis and the effectiveness of treatment. Disease group demonstrated severe pathology associated with +++ cartilage erosion, ++ synovial membrane thickening, and + inflammation of the cells. This observation proves that rheumatoid arthritis has been successfully induced. For standard, full restoration of the joint structure, with the presence of intact cartilage without any signs of inflammation, cartilage erosion, or synovial hyperplasia, showing high chondroprotective and anti-inflammatory activity. Of all the test compounds, the most significant histological improvements have been observed in group NG5 (AKBA nanoemulgel) with partial retention of cartilage and reduced inflammation when compared with the disease control group but there were focal cartilage erosion and synovial thickening.

CONCLUSION

AKBA-loaded nanoemulgel with optimum composition was successfully formulated through the transdermal route using a nanoemulsion system. The formulated nanoemulgel was found to have suitable physicochemical properties and sustained release of the drug along with enhanced skin permeability *ex-vivo*. In addition to that, the *in vivo* studies on pharmacokinetics and dynamics confirmed the suitability of the formulated nanodrug for the better performance of AKBA. The results showed that the nanoemulgel formulation offered superior characteristics in comparison with the conventional gel formulation in terms of drug delivery.

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Conflicts Of Interest:
The authors declare no conflicts of interest

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