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Original Article

Boswellic Acid Loaded Nanoemulgel for Rheumatoid Arthritis: Formulation Design and Optimization by QbD, *in vitro, ex vivo*, and *in vivo* Evaluation

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ABSTRACT

Background: Topical delivery of medication is often considered to be the best choice due to benefits such as avoidance of gastric degradation, first pass metabolism along with effortless application and retrieval. In topical delivery, the types of the vehicles and the physicochemical characteristics of drug are the prime variables that control the drug release and the therapeutic efficacy. The main component of Boswellia, Boswellic Acid (BA), is assumed to be accountable for the majority of pharmacologic effects. Nanoemulgel combines the benefits of nano-emulsions and the gels. **Materials and Methods:** The present work carried out with an objective of formulation of topical nanoemulgel of boswellic acid containing carbopol 934 and liquid paraffin as oil base. **Results:** The prepared nanoemulgel characterized for the various parameters such as, *in vitro, ex vivo* permeation and *in vivo* anti-inflammatory activity. The optimized nanoemulsion consisted of carbopol 934 and surfactant mix ratio (tween 80:Span20::1:1), in 1, 1.5 and 2 % w/w. The *in vitro, ex vivo* permeation of BA from nanoemulgel were found to be 90±0.33% and 85.5±1.2% at 12 hr respectively. **Conclusion:** Topical application of BA nanoemulgel formulation significantly reduced the inflammation by reducing the serum IL-6 concentration compared with the arthritic rats. The research findings showed that BA nanoemulgel has the ability to increase penetration through the skin and produce an anti-inflammatory impact.

Keywords: Boswellic acid, Nanoemulgel, Anti-inflammatory effect, Rheumatoid arthritis, Topical delivery.

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INTRODUCTION

Arthritic illnesses typically include rheumatic diseases, which are the main cause of disability and locomotor dysfunction in humans. Joint discomfort, sleep difficulty, physical incapacity, and lifelong inflammatory consequences are all signs of rheumatoid arthritis.[1] Topical drug delivery, which entails applying medication directly to the skin's surface, is a localized and noninvasive form of administration which reduces adverse effects and systemic absorption,[2] Transdermal delivery of active medicines might be seen as a method for improving skin permeability and prolonged drug delivery, both of which can improve patient compliance.[3,4] Nanoemulsions (NEs), a kind of topical medication delivery method, are appealing on account of their increased skin penetration capacity and low toxicity. Topical nanoemulgels can improve skin permeability with long lasting result at the application site since they contain both gelling agents and NEs.

Gels are generally made by trapping a lot of aqueous phases in a network of colloidal solid particles. Compared to the ointment or cream base, they feature a larger aqueous component that allows for increased drug solubility and easier migration of the drug over a medium. Although gels have several advantages, hydrophobic medicine delivery is a major limitation. Emulgels are created and applied to get over this restriction, allowing hydrophobic drug to take use of gel's special qualities. Emulgel, which is made by combining gels and emulsions, has several beneficial properties such as, greaseless, high spreadability, emollient, non-staining, clear, and pleasant appearance.[5,6] On account of their reduced propensity to produce side effects when compared to synthetic pharmaceuticals, herbal medicines have recently seen a surge in popularity.[7] Emulgels are emulsions (water in oil or oil in water) combined with a gelling agent and shows better drug release and high patient compliance.[8,9] Recent experiments in which pharmaceuticals are put into the cores of nanoemulsions have

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led to increased drug absorption through the skin due to strong subcutaneous barrier penetrability.[10,11]

Boswellic Acid (BA) is a natural product derived from the bark of the boswellia tree. According to reports, BA selectively inhibits 5-lipoxygenase and has anti-inflammatory and antiarthritic property.[12] However, the limited water solubility and short half-life of BA pose significant challenges in the designing of effective topical formulation. As a result, there is a need for the development of an efficacious and safe topical approach for the delivery of BA. Moreover, Boswellic acid's weak water solubility inhibits its skin penetration through the stratum corneum, necessitating the development of a topical administration formulation that increases its permeation through the skin.

MATERIALS AND METHODS

Boswellic Acid obtained as a gift sample from Central India Pharmaceuticals (Nagpur, India). Carbopol-934, Propyleneglycol, span 20 and Tween 80 were obtained from Loba Chemie Ltd. (Mumbai, India). All the ingredients used for the study were of analytical grade.

Formulation of BA Nanoemulgel

The Quality by Design model for the development of dosage form involves methods that commence with objectives set based on formulation understanding and process control to ensure product quality.[13,14] To achieve high *in vitro* drug release and optimal particle size, a 3^2factorial design (three level two factors) was used to statistically optimize the formulation variable. The experimental design was created and evaluated using the Design Expert software version 11 (Stat-Ease Inc.). A total of 9 experiments were run using two independent variables viz. amount of Surfactant mix ratio 1:1 (tween 80: Span 20) in 1, 1.5 and 2% w/w (X1) and ultrasonification time at 2, 4 and 6 min (X2). *In vitro* release (Y1) and Particle size (Y2) were chosen as the dependent variables. The independent variables (Surfactant Mix Ratio-Tween 80:Span 20) in %w/w and dependent variables (% *in vitro* drug release and Particle size (nm) were chosen for the experiment and the composition of the BA loaded nanoemulgel formulation is shown in Tables 1 and 2 respectively. The BA used in the formulation was kept constant.[6]

For the preparation of gel kinase, firstly, Carbopol 934 was dispersed in purified water with continuous stirring using mechanical shaker and Triethanolamine (TEA) then added to maintain pH to 6-6.5. The primary emulsion was prepared by adding oily phase under continuous stirring to an aqueous phase. The gel base was prepared using the remaining water with Carbopol 934 (1% w/w). The prepared BA emulsion then gradually added to the gel phase and homogenized for about 5 min at 10,000 rpm (Homogenize-T-18IKA, Germany). Additionally, it was sonicated using a probe sonicator (PCI-PKS-750 F, India) for different time interval to get desired particle size. BA nano-emulsion was added into the prepared gel base with continuous mixing for 10 min to obtain homogenous nanoemulgel.

Evaluation of the formulations

Physical Characterization

The prepared formulations were visually examined for color, grittiness, homogeneity, clarity, phase separation and consistency.[15]

Determination of pH

After diluting about 1.0 g of the emulgel formulation with 100 mL of distilled water, the pH was measured.[16]

Viscosity

The viscosity of the prepared nanoemulgel was measured using a Brookfield viscometer, (LVDVE, USA) at room temperature.

Spreadability Studies

A modified spreadability apparatus was used to measure the formulations' spreadability. Nanoemulgel (1 g) was placed between two horizontal glass slides (7.5×2.5 cm) and the upper glass slide was applied to a constant load (100 g) for 1 min. Spreadability was measured as the time (sec) required for a moving slide to cover a predetermined distance of 6.5 cm.[17]

Spreadability was calculated as,

$$S=M\times L/T$$

Where, S- Spreadability, M- weight attached to upper slide,

L-Length of glass slides, T-Separation time of slides.

Particle Size

Particle size BA loaded nanoemulgel were determined by using Zetasizer (Malvern Instruments, Worcestershire, UK)

Drug content determination

The BA content of Nanoemulgel formulations was evaluated using UV-visible spectroscopy. Nanoemulgel (1 g) was dissolved in sufficient quantity of phosphate buffer pH of 6.8, filtered, and the volume was adjusted to about 50 mL. An absorbance was measured at 282 nm after appropriate dilution.[18]

In vitro drug release

Drug diffusion investigations were conducted using the modified Franz diffusion cell. Nanoemulgel (1 g) was equally placed to the outer surface of the dialysis membrane, which was positioned between the donor and receptor compartment. Phosphate buffer 6.8 was used as a dissolution medium, and the cell's temperature

was maintained at 37°C. Sample (5.0 mL) was removed at appropriate intervals and replaced with an equivalent quantity of a fresh dissolution medium. Samples were analyzed at 282 nm by using UV-visible spectrophotometer.[19]

Zeta potential

Zeta potential which helps to measure stability is determined by applying an electric field across the dispersion. This method is used to find out charge on drug loaded nanoemulgel using Zetasizer version 6.20 (Malvern Instruments, Malvern, UK). The formulation is considered stable if the zeta potential values observed are more than (+30 mV) or less than (-30 mV).[20] The zeta potential of dispersion is measured by applying an electric field across the dispersion

Field Emission Scanning Electron Microscopy (FE-SEM)

FE-SEM was used to examine the shape and morphology of nanoemulgel (JSM-7610F, Tokyo, Japan). The samples were made by coarsely dusting an adequate amount onto glass slides and then dropping a little drop of the sample on top. These slides were mounted on an aluminium stub and coated with a fine Platinum layer in an argon atmosphere using a cold sputter coater to thickness of 400 A and then photomicrographs were taken at a voltage of 5.0 kV.

Ex vivo studies

Rat dorsal skin using modified franz diffusion cell were used to study skin permeation. To ensure that the skin sample was clear of any surface irregularities (tiny holes or cracks) the skin was thoroughly examined with a magnifying glass. Nanoemulgel (1 g) was equally placed to the surface of the rat skin, which was positioned between the donor and receptor compartments. As a dissolution medium, 6.8 phosphate buffers was used, and the cell's temperature was maintained at 37°C. Sample (5.0 mL) was removed at appropriate intervals and replaced with an equivalent quantity of a fresh dissolution medium. Samples were analysed at 282 nm by using UV-visible spectrophotometer.[21]

In vivo studies

In vivo study of the optimized nanoemulgel was done through formaldehyde induced arthritis model in rats through topical application.[22] Sprague-dawley rats were used to cause arthritis since they develop swelling in many joints under the impact of inflammatory cells, as well as erosion of joint cartilage owing to bone deterioration.[23,24] All animals were handled in accordance with the Institutional Animal Ethics Committee's recommendations (CPCSEA Approval No. 650/PO/Re/S-2002/2022/CPSCEA/31).

Formaldehyde induced arthritis

The animals in the research were separated into 6 groups (n=6). Group-I was given the vehicle (2 mL/kg, 1% v/v) and will act as the normal control. As a negative control, Group II was given formaldehyde. Group III was given the standard drug-marketed diclofenac emulgel (0.5 g topical), whereas Groups IV (0.5 g topical BA Gel), Group V (0.5 g topical BA Nanoemulsion), and Group VI (0.5 g topical BA Nanoemulgel) was given. Arthritis was induced 30 min after administration of drugs/vehicle by

Table 1: 3^2 Full Factorial Design: Factor levels and responses for Nanoemulgel formulation.

Factors (Independent variables)	Factor levels used		
	Low (-1)	Medium (0)	High (+1)
S-Mix Ratio (Tween 80:Span 20)in %w/w (X1).	1	1.5	2
Ultrasonification time in Minutes (X2).	2	4	6
Responses (Dependent variable)			
Y_1=% *in vitro* drug release.			
Y_2= Particle size (nm).			

Table 2: Preparation of Emulgel using following concentration (% w/w).

Ingredient	A1	A2	A3	A4	A5	A6	A7	A8	A9
Boswellic acid	1	1	1	1	1	1	1	1	1
Carbopol 934	1	1	1	1	1	1	1	1	1
S Mix 1:1, (Tween 80:Span 20)	1	1.5	2	1	1.5	2	1	1.5	2
Liquid paraffin	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
PEG 400	5	5	5	5	5	5	5	5	5
Methyl Paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Water (up to)	100	100	100	100	100	100	100	100	100

sub plantar injection of 0.1 mL formaldehyde (2% v/v) into the left hind paw of all animals except the normal control. This was considered as day 1. The drug formulation was used topically for a total of 28 days. On the third day, formaldehyde (0.1 mL 2% v/v) was injected into the same paw. Using a plethysmometer, paw volume and thickness was measured at 0, 7, 14, 21 and 28 days.

Inhibition of paw oedema for untreated groups was calculated using the following formula:

$$i=[1-(\Delta V \text{ treated}/\Delta V \text{ untreated})]x100$$

Where, i=% inhibition of paw edema,

ΔV treated: mean change in paw volume of treated rat,

ΔV untreated: mean change in paw volume of untreated rat.

Arthritis score

The severity of arthritis was assessed by calculating each animal's arthritis index, which was graded from 0 to 4 as previously reported.[25]

Paw volume

The paw volumes of all animals were measured using a plethysmometer (VJ equipment, India) on 0, 7, 14, 21 and 28 days. The difference in paw volume was calculated by subtracting the end and initial paw volumes.[25]

Skin Irritation Test

To evaluate the irritancy of the formulation, male rats were employed in a skin irritation experiment. The animal's dorsal side was clipped one day before the test. Any hypersensitive reaction, including oedema or erythema, was observed at 1, 8, and 24 hr following therapy. The Draize scale was used to assess skin irritancy. The level of irritation was assessed using a scale of 0 (no response) to 4 (severe response).[26]

Biochemical analysis

The blood was collected from retro-orbital plexus to measure the serum TNFα and IL-6levels. For the quantification, serum was separated from plasma and TNFα and IL-6 levels were estimated using commercial ELISA kit as per the manufacturer's protocol. Briefly 100 µL sample and standard were incubated with the antibody followed by series of washing. The levels of TNFα and IL-6 is directly proportional to the color intensity determined using OD value at 450 nm.[25]

Stability study

The Optimized nanoemulgel formulation was packaged in aluminium collapsible tubes (10 g) and stability investigations were performed for 90 days at 40°C±2°C and 75%±5% Relative Humidity (RH). At 30-day intervals, samples were removed and examined for physical appearance, pH, drug content, spreadability, and *in vitro* drug release.

RESULTS

Optimization of the BA Nanoemulgel

The Design Expert® Software was used to develop the optimum formulation, with the goals of maintaining the S-Mix ratio and ultrasonification duration within the specified limits, optimizing cumulative *in vitro* drug release (12 hr), and decreasing particle size. The most desirable ideal formulation was produced and tested for *ex vivo* skin permeability, *in vivo* anti-inflammatory activity, drug content, and physicochemical stability.

Evaluation of the formulations

Physical appearance

The physical characteristics of BA nanoemulgel were tested and are shown in Table 3.

pH Measurement

The pH of all of the formulations was within the intended range, as shown in Table 3.

Drug Content

The drug content was found to be consistent throughout the formulated nanoemulgel with a range of 95.6-99.3%

Spreadability Test

As displayed in Table 3, the spreadability values of BA nanoemulgel formulation were found between 53.21±0.57 mm and 64.6±0.36 mm.[27]

Viscosity Measurement

BA nanoemulgel's viscosity ranged from 5208 to 8128 cP.

In vitro Diffusion studies

The cumulative drug release was found to be between 90.90 and 98.60% (Figure 1).

Experimental design (3^2Full Factorial Design)

The nanoemulgel formulation was optimized using full factorial design (3^2). The formulation variables were used to create all nine batches. RSM was applied in order to assess the effect of % w/w of S-Mix and ultrasonication time as independent variables, as well as their interactions, on the examined responses (% *in vitro* Drug Release and Particle size).

Statistical Analysis and Optimization of Formulation using RSM

As shown in Table 4 the experimental results were evaluated by Design Expert software and mathematical models generated

Table 3: pH, drug content, Spreadability and viscosity of all batches of Emulgel.

Formulation Code	A1	A2	A3	A4	A5	A6	A7	A8	A9
pH	6.32±0.09	6.23±0.07	6.11±0.09	6.37±0.07	6.79±0.05	6.35±0.04	6.12±0.10	6.45±0.03	6.65±0.04
Drug Content	96.1±0.22	96.4±0.13	96.8±0.12	99.3±0.28	98.6±0.34	97.2±0.5	96.2±.31	95.6±0.42	96.6±0.37
Spreadability	54.2±0.41	58.7±0.53	53.7±0.46	63.7±0.56	64.6±0.36	54.44±0.45	58.2±0.55	53.21±0.57	62.2±0.38
Viscosity (cP)	5208±105	5232±132	6126±151	5788±139	6654±161	8128±167	5458±148	6548±143	7908±175

Standard Deviation mean n=3.

for each response to assess the quantitative impacts of variables (A and B) and their levels low (-1), intermediate (0), and high (+1) on the projected responses. The mathematical relationships established using multiple linear regression analysis (MLRA) for the examined response variables dependent variables (% *in vitro* Drug release and% Particle size) that were relating diverse response and independent variables are expressed as the polynomial equations below (quadratic model).

$$Y1\ (DR)=+92.01+2.35A+1.27B+1.05AB+3.48A^2+0.7333B^2 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$Y2\ (PS)=+140.11-28.33A-56.83B+29.75AB-6.67A^2+74.83B^2 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Percent *in vitro* Drug Release (Response 1)

Regression analysis of the aforementioned equation (1) of response Y1 showed that the coefficients of A and B were both positive, indicating that the percentage of *in vitro* drug release increased with increasing S-Mix concentration (A) and ultrasonication time (B), respectively. Contoured and response surface plots were employed to better explain the link between the dependent and independent variables (Figure 2A). The change in % *in vitro* drug release as a function of A and B was depicted using contour and response surface plots based on a full factorial design.

Particle size (Response-2)

The coefficient of A was positive and the coefficient of B was negative in the regression analysis of the above equation (2) of response Y2 (Particle size) as shown in Figure 2B. The particle size of the nanoemulgel formulation was determined to range between 124 and 323 nm.

Determination of Zeta potential

This method is used to determine charge on drug loaded nanoemulgel formulations using zetasizer version 6.20 (Malvern Instruments, Malvern UK). The optimized formulation had shown maximum zeta potential of -24.3 mV.

Morphological Evaluation by FE-SEM

The surface morphology of BA loaded nanoemulgel examined by FE SEM was illustrated in Figure 3.

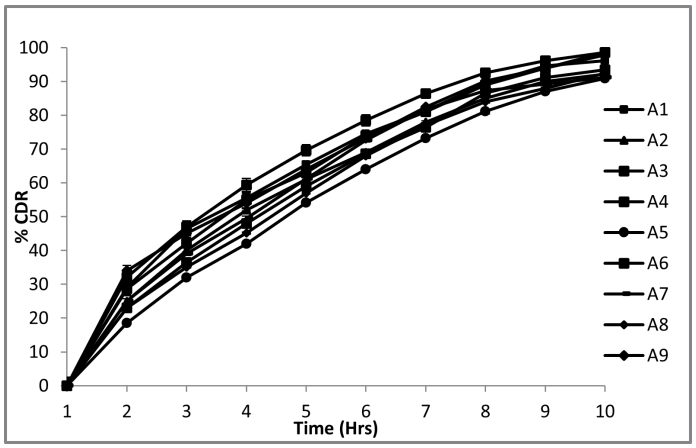

Figure 1: *In vitro* drug release of nanoemulgel formulations.

Ex vivo skin permeation study

The drug permeation across rat skin was evaluated for optimized nanoemulgel preparation (A5) and found to be 85.5±0.29 in 12 hr.

Anti-inflammatory activity

Effect of BA formulation on formaldehyde induced arthritis

The anti-inflammatory effect of BA gel, nanoemulsion, and nanoemulgel was assessed in rats with formaldehyde-induced paw edoema. As shown in Figure 4, the anti-inflammatory potential described by % inhibition of paw volume by BA emulgel formulation was approximately equal to Marketed Diclofenac emulgel (0.5 g, topical), which was used as standard (p<0.001).

Effect of BA formulation of Arthritic score

A significant arthritic score was observed on 21st day as shown in Figure 4.

Skin Irritancy Test

An investigation of skin irritation in rats were carried out by applying a formulation or a vehicle to the dorsal skin. Animal were observed for 24 hr for presence of any skin reaction and scored based on Draize scale.

Effect of BA formulation on serum TNFα and IL-6 concentration

Effect of BA formulation on serum TNF-α (A) and IL-6 (B) concentration depicted Figure 5.

DISCUSSION

The data obtained from the prepared formulations were entered into the Design Expert® Software, which developed models that defined the connection between the independent and dependent variables under consideration. The best fitting models were chosen based on the *p* values and correlation coefficients (R^2) in the individual models. To illustrate the correlations graphically, contour plots and response surface plots were created. The most desirable ideal formulation was produced and tested for *ex vivo* skin permeability, *in vivo* anti-inflammatory activity, drug content, and physicochemical stability. The physical characteristics of BA nanoemulgel showed white, thick and creamy formulations with a homogenous and smooth appearance. pH of all the formulations varied from 6.11±0.09 to 6.79±0.05, which is acceptable and unlikely to cause skin irritation when applied the drug content was found to be consistent throughout the formulated nanoemulgel with a range of 95.6-99.3% indicating that the method employed to prepare the formulation is capable of producing reproducible results. The spreadability values of BA nanoemulgel formulation were found between 53.21±0.57 mm and 64.6±0.36 mm.[27] BA nanoemulgel's viscosity ranged from 5208 to 8128 cP. When all other factors are held constant, this finding supports the literature's theory that surfactant concentration increases formulation viscosity.[28] The *in vitro* release of formulations demonstrated a very fast initial burst, followed by a consistence delayed drug release

Table 4: Composition 3^2 Full Factorial Design with measured responses.

Batch Code	Variable level in coded form		Variable level in actual form		Response Variables	
	Surfactant Mix (%)	Ultrasonification Time (Min)	Surfactant Mix (%)	Ultrasonification Time (Min)	*In vitro* drug release (%)	Particle Size (nm)
A1	-1	-1	1	2	96.2±0.32	323
A2	0	-1	1.5	2	92.2±0.52	293
A3	+1	-1	2	2	98.6±0.21	186
A4	-1	0	1	4	93.5±0.53	149
A5	0	0	1.5	4	90.9±0.38	124
A6	+1	0	2	4	98.6±0.65	134
A7	-1	+1	1	6	91.2±0.44	163
A8	0	+1	1.5	6	92.4±0.54	153
A9	+1	+1	2	6	97.8±0.42	145

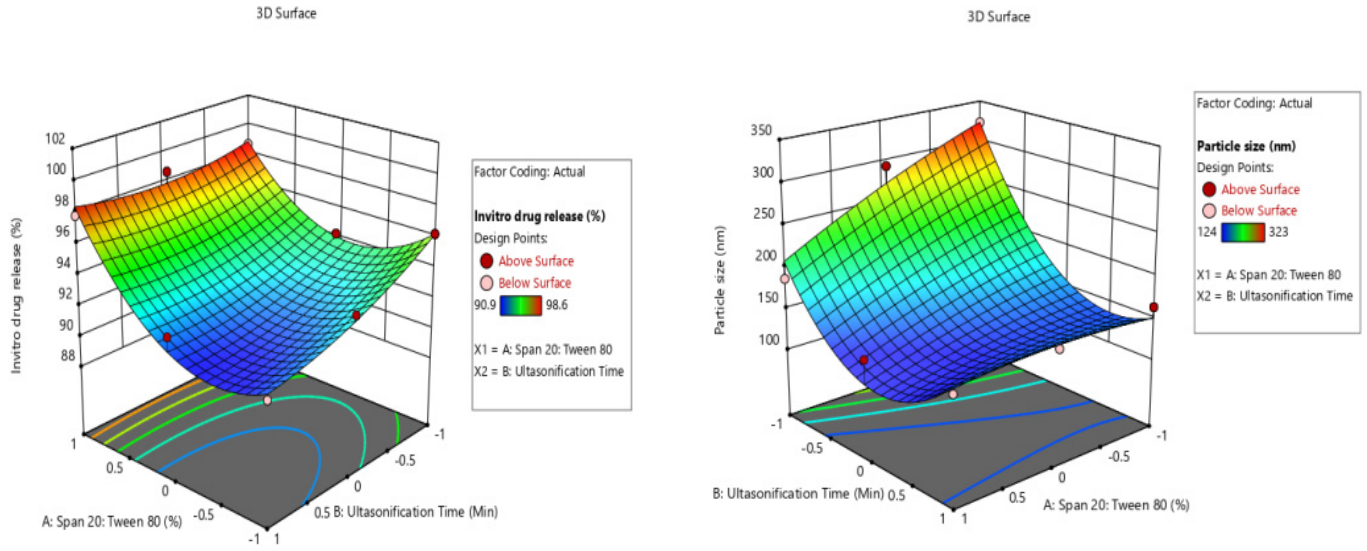

Figure 2: Contour plot and Response surface plot showing the effect of S-Mix (X1) and Ultrasonication Time(X2) on % *in vitro* Drug Release (Y1) and particle size (Y2).

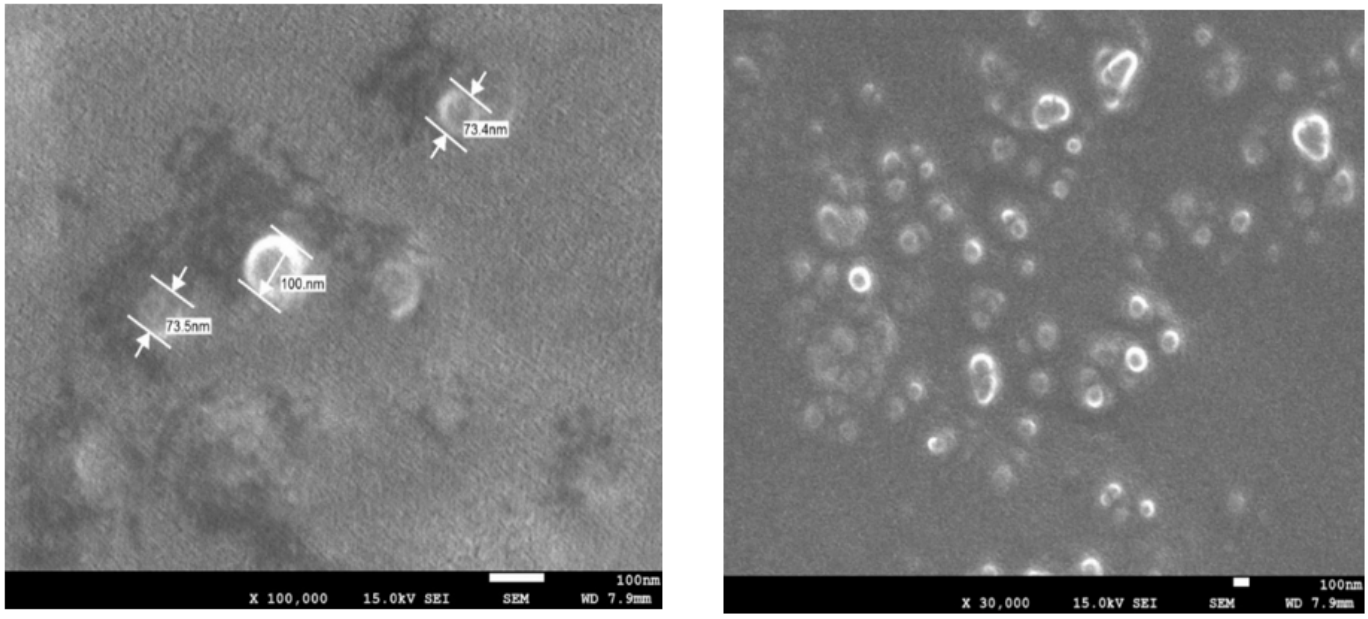

Figure 3: SEM of optimized nanoemulgel formulation.

The experimental results were evaluated by Design Expert software and mathematical models generated for each response to assess the quantitative impacts of variables (A and B) and their levels low (-1), intermediate (0), and high (+1) on the projected responses. Regression analysis of response Y1 showed that the coefficients of A and B were both positive, indicating that the percentage of *in vitro* drug release increased with increasing S-Mix concentration (A) and ultrasonication time (B), respectively. The coefficient of A was positive and the coefficient of B was negative in the regression analysis of the response Y2 (Particle size), suggesting that particle size reduced as the ultrasonification duration increased (Figure 2B). The optimized formulation had shown maximum zeta potential of -24.3 mV. The comparatively high hydrophilic head group of the Tween 80 provides strong repulsive (steric) forces which prevent droplet aggregation. The surface morphology of BA loaded nanoemulgel showed uniform size distribution and spherical shape.

The drug permeation across rat skin was evaluated for optimized nanoemulgel which showed significantly improvement in the rate and amount of drug penetration into rat skin, indicating that it may potentially infiltrate the human skin. The anti-inflammatory effect of BA gel, nanoemulsion, and nanoemulgel was assessed in rats with formaldehyde-induced paw edoema. Significant increase in paw volume was observed with formaldehyde injection from day 1 to day 28 in negative control animals compared with normal control ($F_{interaction}(20,120) = 71.63$, $p<0.001$).Topical application of BA emulgel suppressed the formaldehyde induced inflammation measured by reduction in paw volume compared with negative control ($p<0.001$). The anti-inflammatory potential ascribed by % inhibition of paw volume by BA emulgel formulation was approximately equal to Marketed Diclofenac emulgel (0.5 g, topical), which was used as standard ($p<0.001$). The nanoemulsion formation also showed significant anti-inflammatory activity, whereas application of BA gel failed to produce sustained anti-inflammatory effect compared with nanoemulsion and emulgel. Thus, results shows that the reduction in the paw volume could be attributed to high penetration of BA emulgel through rat skin. Though, the actual mechanism in suppressing the inflammation in formaldehyde induced arthritis is not known, it can be associated with the interaction between inflammatory pathways.

A significant arthritic score was observed on 21st day. Animals treated with BA nanoemulsion and emulgel showed significant reduction in arthritic score from day 7 till end of the experiment compared with negative control ($F_{Interaction}(20,120)=18.04$, $p<0.0001$). The application of BA gel reduced arthritic score during the initial period i.e. till day 14, however was not able to sustained the anti-arthritic effect to the completion of study. Although, both the formulation showed significant reduction in arthritic score, effect of BA emulgel was comparable to diclofenac standard.

The result of skin irritation studies indicated that animals were not allergic to the BA Nanoemulgel formulation. No sign of irritation including erythema or swelling were observed with the formulation in all experimental animals. The overall findings suggest that the produced formulation is safe and well-tolerated for topical use.

There was significant increase in serum TNF-α was observed in arthritic animal as compared with normal control. Application of BA emulgel and nanoemulsion significantly reduced the serum inflammatory marker TNF-α compared with the saline treated

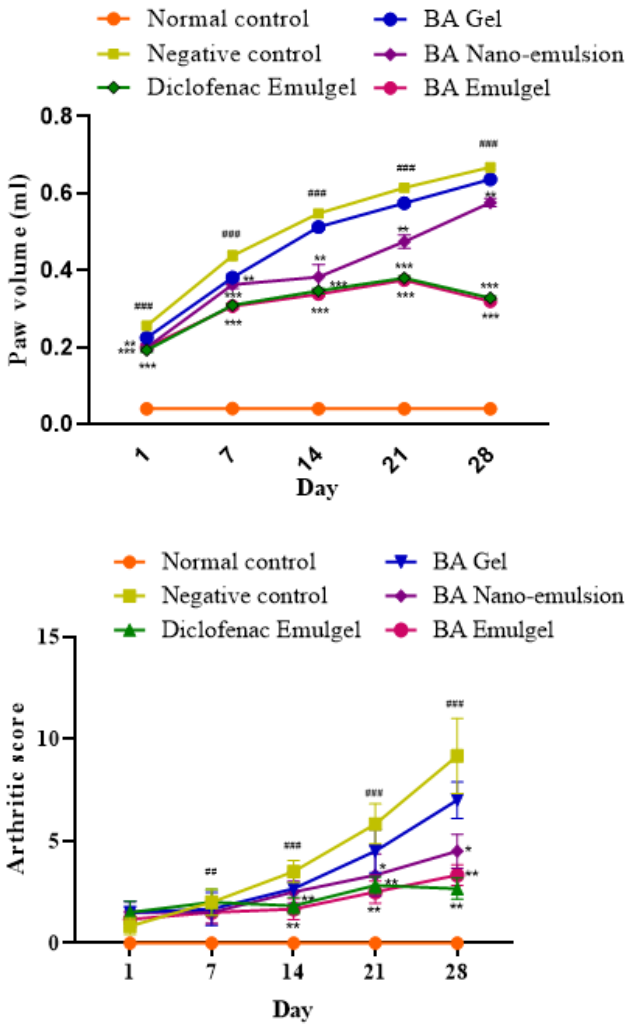

Figure 4: Effect of BA formulation on paw volume.

Each value represents mean±SEM, *n*=6. Two-way analysis followed by post-hoc Bonferroni's multiple comparison tests. ###*p*<0.0001 compared to normal control, **p<0.001, ***p<0.0001 compared to negative control.
Effect of BA formulation on Arthritic score.
Each value represents mean±SEM, *n*=6. Two-way analysis followed by post-hoc Bonferroni's multiple comparison tests. ###p<0.0001 compared to normal control, *p<0.01, **p<0.0001 compared to negative control.

arthritic animals [F(5, 30)=0.5892, p<0.0001]. However, effect of BA emulgel on TNF-α was comparable with standard drug. Also, a significant increase in IL-6 concentration (Figure 5) was observed in formaldehyde induced arthritic animal administered with saline compared with normal control [F (5, 30)=165.8, p<0.0001]. Topical application of BA nanoemulsion and BA nanoemulgel formulation significantly reduced the inflammation by reducing the serum IL-6 concentration compared with the arthritic rats. The effect produced by BA gel was prominent but not comparable to other two formulation (p<0.01).

Additionally, the effect of BA emulgel was approximately equal and comparable with diclofenac. The results shows that the reduction of TNF-α and IL-6 by BA emulgel may be

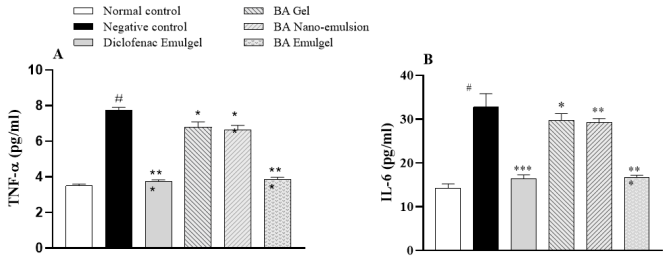

Figure 5: Effect of BA formulation on serum TNF-α (A) and IL-6 (B) concentration.

Each value represents mean±SEM, *n*=6. One-way analysis followed by post-hoc Bonferroni's multiple comparison tests. #p<0.0001 compared to normal control, *p<0.01, **p<0.001, ***p<0.0001 compared to negative control.
Stability Study of optimized BA Nanoemulgel formulation.
According to ICH guidelines, the BA nanoemulgel formulation was subjected to accelerated stability testing. During 90 days, the nanoemulgel was kept at 40±2°C and 75±5% relative humidity.

responsible for reduction of formaldehyde induced arthritis in rats. Consequently, it is probable that the impact of BA emulgel formulation is related to inflammatory pathway reduction by reducing the inflammatory cytokines production such as TNF-α and IL-6.[29] Overall, the findings indicate that the nanoemulgel formulation of BA has the potential to improve skin penetration while also providing an anti-inflammatory impact.

During 90 days, the nanoemulgel was kept at 40±2°C and 75±5% relative humidity. As compared to the initial formulation, the results showed little or no significant phase separation along with no significant changes in appearance, viscosity, spreadability and drug content.

CONCLUSION

Emulgels have grown in popularity in recent years as a topical delivery system that combines the benefits of gels and emulsions. In present research work, a topical nanoemulgel of boswellic acid was developed and characterized for rheological studies, spreadability, *in vitro, ex vivo* drug release studies, and *in vivo* anti-inflammatory activity, which ultimately showed promising results. The findings indicate that the BA nanoemulgel has the potential to improve skin penetration also having an anti-inflammatory impact.

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ETHICAL APPROVAL

Animal procedures were conducted in accordance with the medical research ethics committee (CPCSEA Approval No. 650/PO/Re/S-2002/2022/CPSCEA/31).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BA: Boswellic Acid; **NE:** Nanoemulsion; **IL-6:** Interleukin 6; **TNF-α:** Tumor necrosis factor alpha.

SUMMARY

A topical nanoemulgel with boswellic acid was developed using carbopol 934 and liquid paraffin as the oil base. The formulation underwent characterization for parameters like *in vitro* and *ex vivo* permeation, as well as *in vivo* anti-inflammatory activity. The results showed promise, indicating that the boswellic acid nanoemulgel can enhance skin penetration and exhibit anti-inflammatory effects.

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