Antipsoriatic and Anti-inflammatory Studies of *Berberis aristata* Extract Loaded Nanovesicular Gels

**Nimisha, Dilshad Ali Rizvi**, Zeeshan Fatima, Neema2, Chanchal Deep Kaur3

Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow Campus, Departments of 1Pharmacology and 2Histopathology, Era’s Medical College, Lucknow, Uttar Pradesh, 3Department of Pharmaceutical Sciences, Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India

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**ABSTRACT**

**Objective:** Novel nanovesicular gel of *Berberis aristata* extract was developed and evaluated for its anti-inflammatory and antipsoriatic activity.

**Materials and Methods:** Transfersomes were prepared using soya phosphatidylcholine and edge activators (Tween 80, Span 80, and sodium deoxycholate) by a modified lipid film hydration technique using rotary evaporator and evaluated for various parameters. The quantification and standardization of extract have been carried out using its alkaloid content as berberine as biomarker. Topical application of imiquimod (IMQ) (immune modifier) on the shaved back of mice developed psoriasis-like inflammation followed by histopathological study of inflamed skin. **Results:** The size of transfersomes was in the range of 265–345 nm whereas polydispersity index ranges from 0.10 to 0.63, and for zeta potential, it was from −19.3 to −43.3 mV. Transfersomes were further added to Carbopol 934P for gel formation and subsequently evaluated for their physicochemical properties. Their efficacy against inflammation, IMQ-induced psoriasis, and skin sensitivity was compared with conventional formulation (commercial formulation-Angle Gloss, Phytolab Pvt. Ltd.). Percent inhibition of edema by transferosomal gel (55.76%) was more as compared to conventional gel of extract (33.5%) found out by Carrageenan-induced paw edema method. Primary irritation index was found to be <0.4 inferring its safe use for topical formulation. **Conclusion:** Histopathological report showed that, in psoriasis-induced animal treated with topical application of extract loaded transfersomal gel showed a marked reduction in thickness of epidermis, length of rete ridges as compared to conventional gel formulation. It can be inferred that *B. aristata* extract loaded transfersomal gel can function as potential anti-inflammatory and antipsoriatic formulation.

**Key words:** Anti-inflammatory, berberine, *Berberis aristata*, herbal vesicular gel, transfersomes, vesicles

**SUMMARY**

- The objective of the present research work was to prepare *Berberis aristata* extracts (roots, ethanolic 70%v/v) loaded transferosomal gel, to perform in vitro characterization and in vivo evaluation of their efficacy against inflammation as well as imiquimod (IMQ)-induced psoriasis in animals.
- The remarkable enhancement in the in vitro release efficiency of *B. aristata* extract loaded transferosomal gel resulted in improved anti-inflammatory activity. The prepared novel formulation of *B. aristata* has also shown its efficacy against IMQ-induced psoriasis.

**INTRODUCTION**

Psoriasis can be defined as chronic inflammatory, immune-mediated disorder characterized by marked changes in the skin like rashes, scales, red patches, and itchiness. The inflammatory processes included here may be linked with progression of morbidity and comorbid conditions which severely impair the patient's health.[1]

Histologically, psoriatic skin shows thickened epidermis, absence of granular layer, and epidermal cell nuclei in the superficial layers. A large number of inflammatory cells are present in all layers of the skin.

In psoriasis, the skin becomes rigid due to increase in the level of cholesterol and decrease in ceramides level which leads to a reduction of water content in the skin. This possesses a big challenge to make drug available to the target tissue using topical route.[2] A broad range of therapies are available for treatment of psoriasis; however, their side effects limit their application.[3] It has also been found that, for psoriasis-like inflammatory ailments, a large proportion of population is turning toward herbal remedies with a belief that they are free from any kind of side effects.[4] *Berberis aristata* is one among many herbs that have been widely used in traditional medicines for skin diseases. *B. aristata* is an Indian medicinal plant officially noted in Ayurvedic and Siddha Pharmacopoeia of India,

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which belongs to the family Berberidaceae. It is an ayurvedic herb which is used since ancient times as anti-inflammatory agents for osteoporosis, joint pain, fever, eyes, and skin infection.[9] Topical instillation of aqueous extract of B. aristata showed potent anti-inflammatory activity against endotoxin-induced uveitis in rabbits as well as against turpentine liniment-induced ocular inflammation in rabbit’s eyes.[5,11] Ethnobotanical studies indicate that Rasaut, decoction of B. aristata, was commonly used to treat skin disorders.[9] Literature reports that Malani tribal communities from Himachal Pradesh, India, are also using B. aristata to cure skin disease.[9]

There are many reports existing in literature that emphasize various pharmacological potential of B. aristata extracts, but none of the existing literature reports the conversion of this extract into transferosomal gel and no studies have been done to confirm its antipsoriatic action. Keeping in view the fact that reaching the psoriatic tissue through topical route is difficult and limitation of its existing treatment, novel B. aristata extracts (roots, ethanolic 70%v/v) loaded nanovesicular transferosomal gel have been prepared. The aim of the study was to observe the effect of these prepared nanovesicles on the skin disorders in particular psoriasis.

Imiquimod (IMQ) is a potent immune modifier upon daily spreading on shaved mice skin developed scaly inflamed skin which looks similar to plaque type psoriasis.[10] Distinctive feature of IMQ-induced psoriasis is epidermal hyperplasia, leukocyte infiltration, inflammation, and increased vascularity in dermis.[11] We attempted the mouse model to simulate the features of psoriasis using IMQ.

In the current study, for the quantification and standardization of B. aristata extract, its alkaloid content has been hypothesized as a biomarker.[5,12,13] They have been further evaluated for their efficacy against inflammation as well as IMQ-induced psoriasis in animals.

**MATERIALS AND METHODS**

B. aristata extract was obtained as a gift sample from Aushadhi Herbals New Delhi, India. Berberine chloride dihydrate was obtained as a gift sample from Natural Remedies Pvt. Ltd., Bengaluru. IMQ cream (Glennmark Pharmaceuticals) was purchased from local market. All other solvents and reagents used were of analytical grade.

**Characterization of Berberis aristata extract**

The high-performance thin layer chromatography characterization of B. aristata extract was performed according to our previously described method.[14]

**Preparation of Berberis aristata extract loaded transferosomes**

The transferosome formulations were prepared by modified lipid film hydration technique using rotary evaporator (IKA RV 10 digital).[15,16] Soyaphosphatidylcholine (SPC), edge activator (EA Tween 80/Span 80/sodium deoxycholate), and extract in different ratios were dissolved in 2–5 ml solvent mixture methanol and chloroform (2:1, v/v). The solvent was removed by rotary evaporation under reduced pressure above the lipid transition temperature to form a lipid film on the wall of flask. The thin film was stored overnight for complete evaporation of solvent. The film was then hydrated with phosphate-buffered saline (PBS) 7.4 pH with gentle shaking for 15 min and sonicated for 5 min. The transferosomal dispersion was further hydrated up to 1 h at 2°C–8°C and subjected to evaluation test.[13] Transferosomal dispersions were produced with different SPC:EA ratio (95:05, 85:15, 80:20, and 75:25) and three different EAs (Tween 80, Span 80, and Sodium deoxycholate) [Table 1].

**Evaluation of transferosomes**

All the prepared batches were evaluated for zeta potential (ZP), vesicular size, polydispersity index (PDI), percent entrapment efficiency (EE), and in vitro release study. ZP, vesicle size, and PDI were measured by Zetasizer (Malvern Instruments, MAL 1021334, version 7.03).

**Entrapment efficiency**

Aliquots of transferosomal dispersions were subjected to centrifugation using cooling centrifuge (Remi C-24BL) at 12000 rpm. The clear supernatant was pipetted off carefully to separate the unentrapped extract. Sediment was treated with 1 ml of 0.1% Triton X-100 to lyse the vesicles and then diluted with PBS (pH 7.4). The EE was determined in terms of percentage berberine (BE) content in sediment. Since BE is one of the major and important alkaloids found in B. aristata which is responsible for its pharmacological property;[12] therefore, it was considered as a biomarker for estimations.[18,19]

The percent entrapment was calculated using the formula:

Percent Entrapment efficiency = (amount of BE in sediment)/(amount of BE in the extract added to transferosome) × 100.

Visualization of vesicles by phase contrast microscopy and transmission electron microscopy (TEM).

All the prepared batches were observed under phase contrast microscopy with the magnification power of ×100 (Olympus). Photographs of vesicles were taken using Olympus camera (Olympus MJU 9010). Optimized batch was visualized using Jeol/JM 2100, Source LaB6 electron microscope (TEM) with an accelerating voltage of 200 kV for surface appearance and shape. The transferosomes were dispersed in distilled water and 10 μl of diluted dispersion was placed on carbon-coated grid.

**In vitro diffusion studies**

The dialysis membrane was placed in PBS (7.4 was selected because high release rate was obtained when compared to pH 5.4 and pH 6.8) for 6 h to attain saturation before starting permeation study. It was mounted between the donor and receptor compartment of the Franz diffusion cell (fabricated with glass) for in vitro diffusion studies. Transferosomal dispersion to be analyzed was placed into the donor cell compartment. The receptor chamber was filled with PBS (7.4) and was maintained at 37°C with continuous stirring. One milliliter aliquot of receptor phase solution was withdrawn at time intervals of 0.5, 1, 2, 4, 8, and 24 h, and the same volume of fresh medium was added back into the chamber. The quantification was done using ultraviolet spectrophotometer (Shimadzo Model No. 1800) at 203 nm.

| Table 1: Formulation table of Berberis aristata extract loaded transferosomes |
|-----------------------------|-------|-----------|-------------|---|
| Formulation codes | EA | PCEA ratio | Extract quantity (mg) |
| T1 | Tween 80 | 95:05 | 10 |
| T2 | Tween 80 | 85:15 | 10 |
| T3 | Tween 80 | 80:20 | 10 |
| T4 | Tween 80 | 75:25 | 10 |
| S5 | Span 80 | 95:05 | 10 |
| S6 | Span 80 | 85:15 | 10 |
| S7 | Span 80 | 80:20 | 10 |
| S8 | Span 80 | 75:25 | 10 |
| SD9 | Sodium deoxycholate | 95:05 | 10 |
| SD10 | Sodium deoxycholate | 85:15 | 10 |
| SD11 | Sodium deoxycholate | 80:20 | 10 |
| SD12 | Sodium deoxycholate | 75:25 | 10 |

EA: Edge activator; PC: Phosphatidylcholine.
Permeation data analysis
To study the release rate profile, the data obtained from in vitro drug release study were fitted in different kinetic equations: zero order as the cumulative percent of drug remaining versus time, first order as the log cumulative percentage of drug remaining versus time, Higuchi’s model as the cumulative percent drug remaining versus square root of time, Hixson-Crowell cube root model and Korsmeyer–Peppas model as the log cumulative percentage of drug released versus log time.

Vesicle penetration study by fluorescence microscopy
The ability of fluorescence marker-loaded transferosome to penetrate through skin was observed by fluorescence microscopy. Franz diffusion cell was used to perform the study in a similar manner to the in vitro diffusion studies. The fluorescent probe rhodamine B was loaded in the vesicles along with extract during the hydration of the film with PBS 7.4 pH with gentle shaking for 15 min. Probe loaded vesicular dispersions were applied for 8 h to the dorsal skin of rats in Franz diffusion cell at 37°C. At the end of the experiment, skin was removed from cell and thoroughly washed with distilled water. Treated area was cut out from the skin and sectioned into 20 μ thickness tissues using cryotome (Thermo Scientific HM550). It is highly efficient instrument used for sectioning technique in routine and research. Skin specimens were observed under fluorescence microscope Leica DM2500, using Green Filter N2.1, position 3. The skin specimens (20 μm) were placed on glass slide irradiated with 540 nm and observed at magnification of ×100.

Incorporation into hydrophilic gels
On the basis of EE, percentage drug release, PDI, and ZP, four transferosomal batches (T3, S6, S7, and SD11) were selected for incorporation into gel formulations. Carbopol 934P 0.5% w/v was soaked in a minimum amount of water for an hour, and then 10 ml of transferosomal dispersion containing B. aristata extract (10 mg) was added. It was then stirred continuously at 700 rpm in a closed vessel whose temperature was maintained at 30°C until homogeneous transferosomal gels was achieved. To maintain the neutral pH, triethanolamine was added slowly to the stirring mixture.

Evaluation of gel-viscosity and pH measurement
Viscosity of transferosomal gel formulations were measured using Brookfield viscometer (Model No. DV-III ULTRA) using spindle no. 6 at 100 rpm and pH measurements of the formulations were done using digital pH meter (Model RI-152-R).

Spreading diameter
The spreadability of gel formulation was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) after 1 min. The standard weight applied on upper plate was 125 g.[20,21]

Drug content of the formed gels
Five hundred micrograms of gel was taken and dissolved in 50 ml of pH 7.4 PBS. The solution was then passed through the filter paper and 50 μl of the filtrate was withdrawn. The filtrate was diluted by adding 3.5 ml of distilled water and the drug content was measured spectrophotometrically at 203 nm (extract) against corresponding gel concentration.[20,21]

In vitro diffusion studies
The in vitro study for transferosomal gel was performed in the same way as it was carried out for transferosomes.

Experimental animals for psoriasis
Five-week-old male mice with no previous drug treatment were used for in vivo studies. The animals were fed on standard diet and water ad libitum. Acclimatization to laboratory hygienic condition was done for 7 days before the starting experiment. Twenty-four mice were taken and divided into four groups (G1–G4) six animals in each group. Hairs on the dorsal portion of each mouse were removed using depilatory cream. The animal experimentation was performed according to Institutional Animal Ethical Committee of Era’s Lucknow Medical College, Lucknow (Registration Number 1652/PO/a/12/CPCSEA).

Induction of psoriasis
This method was a slight modification from the study design by van der Fits.[20] The animals were shaved on the dorsal skin. Control group animals (G1) were treated with 50 mg of Vaseline on the dorsal skin surface for 11 days. IMQ cream (Glenmark Pharmaceuticals) 50 mg was applied topically on the dorsal skin surface of each mouse of Group 2–Group 4 to induce psoriasis once daily for 11 days.[21,22]

Treatment of psoriasis
B. aristata extract loaded transferosomal gel formulation (50 mg) was applied topically on Group 2 animals daily for 12 days. Its conventional gel formulation (50 mg) was applied to Group 3 animals. Group 4 animals were left untreated. In the control group, animals’ application of Vaseline was continued. Body weight, food, and water intake of each animal were recorded daily.

Histological studies
The dorsal skin was excised from the mouse at the end of experiment. The skin samples were immersed in 10% formalin, embedded in paraffin wax, and sliced at a thickness of 3 μm using microtome (Leica RM2245). The samples were stained by hemoxlyin and eosin (H and E) and observed under microscopy (Leica DM2500). Photographs of ×40 magnification of H- and E-stained skin sections of normal, disease-induced, disease-induced animal treated with B. aristata extract loaded transferosomal gel formulation, and disease-induced animal treated with conventional gel formulation were taken.

Experimental animals for anti-inflammatory study
Wistar rats of either sex (150–200 g) with no previous drug treatment were used for in vivo studies. The animals were fed on a standard diet and water ad libitum. Acclimatization to laboratory hygienic condition was done for 7 days before the starting experiment. The animal experimentation was performed according to Institutional Animal Ethical Committee of Era’s Lucknow Medical College, Lucknow (Registration Number 1652/PO/a/12/CPCSEA).

Carrageenan-induced paw edema
Carrageenan-induced paw edema method was used for anti-inflammatory evaluation. Wistar albino rats were divided into three groups (n = 6). The first (control) and second (positive control) groups were treated with carbopol base gel and extract loaded transferosomal gel (optimized batch). Third group animals were treated with conventional gel. All the treatments were given to the planter surface of the left hind paw of rats by gentle rubbing of 0.5 g of gel with the index finger after 60 min of induction of edema. Edema was induced by injecting 0.1 ml of Carrageenan (1% w/v) in normal saline into the treated paws of all the rats. Paw edema was measured 0 h, 1, 2, 3, and 4 h after injection of Carrageenan using plethysmograph.
The percent of inhibition of edema was calculated using the following formula:

\[
\% \text{inhibition of edema} = \left(1 - \frac{V_t}{V_c}\right) \times 100
\]

Where \( V_t \) is mean volume of paw edema in drug-treated group, \( V_c \) is mean volume of paw edema in control group.

**Skin irritation study**

Wistar rats of either sex (150–200 g) with no previous drug treatment were used for in vivo studies. The animals were fed on a standard diet and water ad libitum.

All animals were divided into three groups \( (n = 6) \). Group 1 (control) animals were treated with base gel. B. aristata loaded transferosomal gel (optimized batch) and conventional gel were applied on Group 2 and 3 animals, respectively. Skin irritation test was done according to the method of Draize. Treated area was covered with gauze and wrapped with nonocclusive bandage. The bandage and test material were removed after 24 h and it was observed at 24 h and 48 h for erythema and edema.\(^{[23]}\)

**Statistical analysis**

Differences in the in vitro release profile of prepared formulations were tested for significance using independent t-test using SPSS version 12.0. Difference was considered significant when \( P < 0.05 \). Graphs were prepared using GraphPad Prism 3 (GraphPad Software, Inc.).

**RESULTS**

**High-performance thin layer chromatography**

The presence of a specific peak for BE at Rf around 0.38 was recorded and considered as a positive result for B. aristata extract as already reported in our publication.\(^{[14]}\)

**Morphology**

The phase contrast micrographs and TEM of optimized batch showed the surface morphology of transferosomes [Figure 1a and b].

**Vesicle size, polydispersity index, and zeta potential**

The size of transfersomes is in between 265 and 345 nm whereas PDI ranges from 0.10 to 0.63 and its ZP lies from −19.3 to −43.3 mV. The results are presented in Table 2.

**Entrapment efficiency**

The range of EE of transfersomes was between 42.5% and 90.01% [Table 2].

**Physicochemical properties of gel**

The results of all the physicochemical properties of the transferosomal gels are presented in Table 3.

**Drug content**

The drug content of the gels ranged between 78% and 94% was maximum in S7 formulation and minimum in SD11 [Table 4].

**Vesicle penetration study by fluorescence microscopy**

Probe loaded transferosomal dispersions were able to permeate to deeper layers of the skin and this was confirmed by the presence of fluorescence in the skin specimens after analyzing under fluorescence microscope Leica DM2500, using Green Filter N2.1, position 3.

**In vitro diffusion studies**

Percent cumulative amount of drug release in 24 h ranges from 76% to 99% for transferosomal gel whereas only 40% for pure extract. In vitro drug release studies transferosomal gel formulations (T3, S6, S7, and SD11) and conventional gel were showed that cumulative percent drug release for B. aristata extract loaded transferosomal gel is significantly higher than \( (P < 0.5) \) B. aristata extract loaded conventional gel.

**Anti-inflammatory activity and skin irritation test**

Intraplantar administration of Carragenan in the right hind paw of rats produced edema. B. aristata extract was able to produce a significant reduction in paw volume when compared with control. Percent inhibition of edema by conventional gel of extract (33.5%) was significantly less than transferosomal gel (optimized batch-55.76%). Primary irritation index for both the gel formulation comes out to be <0.4 which shows negligible irritation and the preparations are safe for topical use [Table 4].

**Histopathological observations for psoriatic skin**

Compared with the control, dorsal skin sections of disease-induced animal showed increased epidermal thickness, elongation of rete ridges, and capillary loop dilation. This confirms the successful development of disease-induced animal model. Topical application of B. aristata extract loaded transferosomal gel formulation showed a marked reduction in thickness of epidermis.

**Table 2: Entrapment efficiency, vesicle size, polydispersity index, and zeta potential of the various transferosomal preparations**

| Formulation codes | Entrapment efficiency (%) | Vesicle size (nm) | PDI | Zeta potential (mV) |
|-------------------|--------------------------|------------------|-----|-------------------|
| T1                | 57.5±1.82                | 302±12           | 0.44±0.052 | −39.0           |
| T2                | 62±1.14                  | 295±14           | 0.63±0.038 | −39.2           |
| T3                | 79.4±0.8                 | 288±13           | 0.56±0.042 | −40.2           |
| T4                | 70.5±0.8                 | 270±8            | 0.39±0.052 | −35.6           |
| S5                | 42.5±2.03                | 345±15           | 0.41±0.016 | −38.2           |
| S6                | 78.0±1.12                | 330±19           | 0.29±0.007 | −35.2           |
| S7                | 90.0±1.16                | 295±12           | 0.10±0.052 | −43.3           |
| S8                | 77.0±0.8                 | 275±10           | 0.47±0.052 | −36.2           |
| SD9               | 59.0±0.8                 | 300±11           | 0.37±0.003 | −41.1           |
| SD10              | 70.0±2.11                | 290±13           | 0.26±0.013 | −19.3           |
| SD11              | 76.7±1.83                | 285±14           | 0.39±0.013 | −28.9           |
| SD12              | 68.1±4.80                | 265±11           | 0.46±0.052 | −35.0           |

All the values are expressed as mean±SD. PDI: Polydispersity Index; SD: Standard deviation
less elongation of rete ridges with capillary loop dilation. However, conventional gel formulation-treated animal showed nominal decrease in epidermal thickness [Figure 2 a-d].

DISCUSSION

Transdermal delivery is most preferred and commonly used for psoriasis as it bypasses first-pass metabolism. The challenge to conventional topical delivery is its transport across the skin barrier.

In psoriasis, the condition is more complicated because the rigid and dehydrated skin poses more resistance. To overcome this problem, Mental et al., 2009, proposed the delivery of drugs through lipoidal carrier systems.

Transfersomes are one such nanovesicular lipoidal carrier which is being widely used for transdermal delivery. They easily pass through the skin and increase the accumulation of drug in the skin. Ghanbarzadeh and Arami, 2013, had reported that the steady state transdermal rate of diclofenac sodium transfersomes is increased 2–3 times as compared to normal liposome. It has also been reported that, compared to vesicular dispersions, incorporation of vesicular formulation into gel preparation could significantly increase flux and Kp up to 3 times (P < 0.05). In the present study, B. aristata extract

Table 3: Evaluation of physicochemical properties of Berberis aristata extract loaded transferosomal gel

| Formulation | Color          | Homogeneity | Texture | Viscosity (centipoise) | pH | Spreading diameter after 1 min (mm) | Drug content (%) |
|-------------|----------------|-------------|---------|------------------------|----|------------------------------------|-----------------|
| T3          | Yellowish white| Homogenous   | Smooth  | 4800                   | 6.2| 55                                 | 78.4            |
| S6          | Yellowish white| Homogenous   | Smooth  | 4500                   | 6.7| 48                                 | 78              |
| S7          | Yellowish white| Homogenous   | Smooth  | 4700                   | 7.0| 42                                 | 94.34           |
| SD11        | Yellowish white| Homogenous   | Smooth  | 4600                   | 6.6| 44                                 | 70.75           |

Table 4: Evaluation of anti-inflammatory activity of Berberis aristata extract loaded transferosomal and conventional gel

| Groups (treatments) | Paw edema | Percent edema inhibition |
|---------------------|-----------|--------------------------|
|                     | 0 h       | 1 h                      | 2 h | 3 h | 4 h | 1 h | 2 h | 3 h | 4 h |
| I                   | 0.504±0.024| 0.507±0.024 | 0.667±0.040 | 0.813±0.036 | 0.728±0.019 | -   | -   | -   |
| II                  | 0.412±0.018| 0.412±0.018 | 0.528±0.014 | 0.485±0.018 | 0.480±0.013 | 18.73 | 20.83 | 40.34 | 33.5 |
| III                 | 0.394±0.012| 0.394±0.012 | 0.411±0.013 | 0.048±0.013 | 0.322±0.016 | 22.28 | 38.38 | 47.35 | 55.76 |

Values are expressed as mean±SD of n=5. SD: Standard deviation

Table 5: Kinetic assessment of release profile of Berberis aristata extract loaded transferosomal gel

| Formulation codes | Zero order model ($r^2$) | First order model ($r^2$) | Higuchi model ($r^2$) | Hixson-Crowell model | Korsmeyer and Peppas model, r ($n$) |
|-------------------|--------------------------|---------------------------|----------------------|----------------------|-------------------------------------|
| T3                | 0.55                     | 0.92                      | 0.815                | 0.806                | -                                   |
| S6                | 0.708                    | 0.876                     | 0.918                | 0.824                | 0.829                               |
| S7                | 0.559                    | 0.972                     | 0.823                | 0.962                | -                                   |
| SD11              | 0.759                    | 0.994                     | 0.946                | 0.955                | 1.32                                |
| Conventional gel (Berberis aristata) | 0.827 | 0.876 | 0.922 | 0.860 | 1.24 |
loaded transfersomal formulations were prepared for the treatment of psoriasis and inflammation.

Size and shape of the vesicle is a crucial factor in the therapeutic performance of transdermal drug delivery systems. Phase contrast microscopy showed the surface morphology of transfersomes [Figure 1a]. TEM of optimized batch (S7) showed spherical vesicles [Figure 1b]. The structural image showed a light center space and enclosed by a dark heavy boundary which is completely intact.

In general, particle size increased along with surfactants possessing lower hydrophilic-lipophilic balance (HLB). Surfactants interact with lipid head groups in the membrane and increase packing density of layer, leading to high surface free energy. The HLB value of these surfactants such as Span 80, Tween 80, and sodium deoxycholate are 4.3, 15, and 16, respectively. Their affinity with lipids is in the order of S>T>SD.

Since Span 80 has highest affinity with lipids, it resulted in expanded size of Span 80 transfersomes. However, no significant difference in size between transfersomal formulations containing Tween 80 and sodium deoxycholate was observed. The size of the vesicles was also affected by EA and phospholipids concentration.

It was also found that vesicle size of B. aristata extract loaded transfersomes decreased with increase in EA concentration and increased with increase in phospholipid concentration.

PDI was considered for evaluation of homogeneity of prepared transfersomes on the basis of their particle size distribution. The transfersomal preparations showing PDI between 0 and 1 were selected [Table 2].

Stability of transfersomal formulation can be assessed by its ZP. The negative ZP is responsible for enhanced percutaneous permeation of drug. All the transfersomal formulations were found to have negative ZP (−19.3 to −43.3 mV) due to the net charge of the lipid composition in the formulation.

The EE in transfersomes depends on the EA concentration in the lipid bilayer. Transfersomal formulations containing varying percent of Span

![Figure 4](https://example.com/image.png)

**Figure 4:** (a) The zeta potential of Batch S7. (b) The size and size distribution of Batch S7 (reproduction size at column width)
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in vitro

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Pharmacognosy Magazine, showed that cumulative percent drug release for gel were also evaluated for their EE. The EE of T1 to T4 was found to be 57.5%–70.5% whereas it was 42.5%–77.03% for S5 to S8 and, for SD9 to SD 12, it ranges between 49.01% and 68.14%.

Initially, with increasing EA (5%–20%), there was an increase in EE, but it was observed that above 20% concentration of EA EE decreased. This may be due to micelles formation in the vesicle membranes at higher EA concentration. This corroborates with the report which states that micelles have a lower drug carrying capacity and poor skin permeation due to their internal structural feature.

On the basis of EE, percentage drug release, PDI, and ZP four transferosomal batches (T3, S6, S7, and SD11) were selected for in vitro release profile as well as incorporation into gel formulations.

Four batches (S6, S7, SD11, and T3) of transferosomal dispersion and pure extract were subjected to in vitro diffusion studies.

The cumulative amount of drug release was calculated for each formulation, and it was found that percent cumulative amount of drug release in 24 h ranges from 76% to 99% for the respective batches taken which was significantly higher than for pure extract (P < 0.05) [Figure 3].

Among the four selected transferosomal batches, batch S7 (Span 80, 20%) showed maximum drug release up to 24 h, may be due to highest EE. This is also in accordance with the results reported by Agarwal et al., which states that, at optimum EA concentration, (20%) the EA molecules get linked with SPC bilayer, thus enabling partitioning of drug across the vesicle easily and higher percentage drug release. The batch also depicted EE, vesicle size, ZP, and PDI as 90.01%, 295 nm, -43.3, and 0.10, respectively, so it was selected as an optimized batch [Figure 4a and b].

Transferosomes with composition (PC:EA ratio 80:20, extract quantity 10 mg) and fluorescent probe rhodamine B 0.03% were prepared and were subjected to vesicle penetration study by fluorescence microscopy. Probe loaded transferosomal dispersions were able to permeate to deeper layers of the skin and this was confirmed by the presence of fluorescence in the skin specimens (20 μm) after analyzing under fluorescence microscope Leica DM2500, using Green Filter N2.1, position 3 [Figure 5].

The prepared gels were evaluated for physical appearance, pH, spreadability, viscosity, and drug content. Gels were found to be smooth, homogenous, yellowish white in color, pH lying in the normal skin pH range, easily spreadable, and viscosity ranging between 4500 and 4800 cps [Table 3].

Transferosomal gel formulations (T3, S6, S7, and SD11) and conventional gel were also evaluated for their in vitro drug release studies. Results showed that cumulative percent drug release for B. aristata extract loaded transferosomal gel is significantly higher than (P < 0.5) B. aristata extract loaded conventional gel [Figure 6].

These results suggested that transferosomal gel can enhance the skin permeation of drug.

The data obtained from in vitro release study of transferosomal gel and conventional gel was fitted in different kinetic equations to access the release rate profile. Both the batches S6 and SD11 were best fitted for the Higuchi kinetic model as the r² value was higher than other values. This implies slow and steady release by the process of diffusion as proposed by Higuchi. Batch S7 showed first-order release kinetics inferring that the rate of diffusion is directly proportional to concentration of drug [Table 5].

Anti-inflammatory activity and skin irritation study was done for optimized batch transferosomal gel (Batch S7) and conventional gel. Percent inhibition of edema for Batch S7 transferosomal gel was 55.76% as compared to conventional gel which showed only 33.5% inhibition. This study implies that transferosomal gel has greater potential to decrease inflammation. Primary irritation was <0.4 indicating negligible irritation in all the three groups. Hence, the developed formulations are free from skin irritation. Histopathological study revealed that topical application of B. aristata extract loaded transferosomal gel formulation causes a marked reduction in thickness of epidermis and elongation of rete ridges than compared to conventional gel formulation in psoriasis-induced animal. This may be due to enhanced permeation ability of B. aristata extract loaded transferosomal gel through psoriatic skin [Figure 2a-d, and Table 4].

CONCLUSION

Plants have always been a component of mankind’s health-care system. There is great potential in herbal drug candidates although very few examples exist in literature that emphasize on novel delivery system bearing herbal extracts and phytoconstituents. Novel herbal delivery systems not only increase the therapeutic efficacy by reducing toxicity but also increase bioavailability. The aim of the present study was to prepare transferosomes of herbal extract of B. aristata for treating inflammation and psoriasis. The remarkable enhancement in the in vitro release efficiency of B. aristata extract loaded transferosomal gel resulted in improved anti-inflammatory activity. The prepared novel formulation of B. aristata has also shown its efficacy against IMQ-induced psoriasis. We

**Figure 5:** Fluorescence micrograph image (reproduction size at column width)

**Figure 6:** Comparative in vitro release profile of transferosomal gel (T3, S6, S7, and SD11) preparations and conventional gel (reproduction size at column width)
conclude that this system may be a better therapeutic agent as compared to the conventional system for psoriasis as well as inflammation. However, further biological and clinical studies have to be carried out to establish the mechanism of action.

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**Conflicts of interest**

There are no conflicts of interest.

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