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Andrographolide nano-invasomal gel: formulation, characterization, and ex vivo study for improved transdermal delivery

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ABSTRACT

The *Andrographis paniculata* plant produces andrographolide, a labdane diterpenoid that has a wide range of therapeutic uses. However, because of its low skin penetration and limited water solubility, andrographolide transdermal distribution and performance face significant challenges. Invasomes containing andrographolide were created utilizing a perfunctory dispersal procedure. Invasomes were identified and improved in expressions of skin retention studies, vesicle size, ex vivo permeability, entrapment efficiency, and in vitro drug release. The ideal invasomal formulation was turned into a topical gel. The Higuchi kinetic model for release was strongly supported by the invasomal gel *in vitro* release studies. The invasomal gel's andrographolide penetration through the skin of the pig's ear was 2.5 times greater and had a flow of 155.020.12g/cm^2/h, according to an ex vivo research. According to the study's findings, invasomes have a high potential for delivering andrographolide transdermally, which could increase andrographolide's topical utility in a variety of skin disorders.

Keywords: Andrographolide, Labdane diterpenoid, Nano-invasomes, Skin, *Ex vivo* study, Transdermal

Introduction

The primary biologically active component of *Andrographis paniculata* is andrographolide (AG), a naturally occurring diterpenoid. Among the effects listed include immune system activation[1], preventing the onset of psoriasis, and antibacterial and antiviral properties[2]. The primary biological effects include those that are anti-inflammation, anti-hyperglycaemic, anti-hepatoprotective, anti-viral, anti-bacterial, anti-fertility, and anti-cancer. As a result, it is used in Ayurvedic and traditional Chinese medicine. By preventing I-phosphorylation B's in the NF-B pathway and down regulation of inflammation, pulmonary administration of liposomal AG powder ameliorated bacterial pneumonia[3]. Uncertain molecular processes underlie AG action. The majority of studies noted NF-kB activation suppression. Others, however, have shown modifications to the PI3K and MAPK pathways as well as a suppression of NFAT activation[4]. Even though significant biological activity has been repeatedly shown, AG's low solubility and poor selectivity make it difficult to utilize in pharmacology and medicine, which has led to attempts to change its structure and the creation of a number of analogues. Recently, low levels of AG in various extracts have been increased by the application of several inducers[5], but the outcomes are not yet adequate. With far more percutaneous permeation than typical liposomes, terpenes, ethanol, phosphatidylcholine, or a mixture of terpenes make up the flexible phospholipid-based nanoscale vesicular structures known as invadesomes[6, 7]. Terpenes are powerful permeation enhancers that quickly alter the stratum corneum's packing, interact with intracellular proteins, disrupt its lipid structure, and significantly improve stratum corneum drug partitioning[7,8]. Ethanol and the terpenes in invasomes work together to facilitate invasomal vesicle penetration through the skin[9-11]. Invasomes provide a number of benefits, including non-invasive drug delivery, enhanced skin absorption of pharmaceuticals, distribution of equally hydrophilic and lipophilic medications, and higher patient fulfillment due to the drug's availability in cream or semisolid gel form. A limited capacity to dissolve in water and a low level of permeability of AG, despite its many benefits and therapeutic importance, seriously jeopardise its transdermal absorption and effectiveness. The study's aim was to enhance topical delivery of AG through nanosized invasomal vesicles, resulting in better AG transport through the skin for higher therapeutic action.

Materials and methods

Chemical reagents

The sources for andrographolide and carbopol 934 were Sigma-Aldrich Chemical Co. in Mumbai, India and Milwaukee, Wisconsin, USA. We purchased soy lecithin and pure ethanol from SD Fine-Chem Ltd. in Mumbai, India. The supplier of nerolidol was Hi Media Laboratories Pvt. Ltd. The study's chemicals and solvent were of analytical grade.

Andrographolide invasomes preparation

The AG invasomes were created using a mechanical dispersion approach. In a nutshell, soya phosphatidylcholine was combined with ethanol, vortexed for 5 minutes to create an apparent solution, and then subjected to 5 minutes of sonication. With the aid of a syringe, a delicate stream of decanted water (up to 10% v/v) was continuously injected into the produced combination while vortexing. To get the finishing invasomal formulation, vortexing was maintained for an extra five minutes (Table 1) [12-15].

TABLE 1: Composition of different invasomal formulation

Formulation	Drug (w/v)	Terpene (w/v)	Ethanol (v/v)	Polymer (w/v)
N1	1.5%	0.1%	10%	1.2%
N2	1.5%	0.5%	10%	1.2%
N3	1.5%	1%	10%	1.2%
N4	1.5%	1.5%	10%	1.2%

Characterization of AG loaded invasomes

% EE (Entrapment efficiency)

For measuring the per entrapment of the invasomal formulation, ultracentrifugation was performed. The invasive formulation was centrifuged at 15,000 rpm for 40 min in an ultracentrifuge. The obtained sediment was further diluted with ethanol after being collected. The AG content was examined using UV spectrophotometry at a wavelength of 224 nm[16, 17]. The following equation was used to get the % EE:

$$\% EE = amount\ of\ entrapped\ drug\ recovered / total\ amount\ of\ drug \times 100$$

Particle size

The vesicles' sizes were measured using a zeta sizer (Nano- ZS, Malvern, U.K.). Each experiment was performed three times[18].

Release of drugs in vitro

The receiver cell volume and effective permeation area used for the in vitro drug release experiment were 10 ml and 0.196 cm2, respectively, in Franz's diffusion cell. The donor cell with the invasomal formulation was clamped over the receptor cell that was filled with phosphate buffered saline (pH 7.4). The experiment was run for 24 hours at a temperature of 37°C with a constant magnetic stirring speed of 600 rpm. The amount of AG in samples obtained from the receptor cell at specific times1, 2, 3, 4, 5, 6, and 24h was determined using a UV spectrophotometer set at 224 nm. To ascertain the release kinetics of the enhanced invasomal formulation, several release kinetics models were used to the data[19, 20].

Ex-vivo investigations on drug permeation

The ex-vivo drug permeation study utilised Franz's diffusion cell, with receiver cell volume and effective permeation area of 10 ml and 0.196 cm2 respectively. The amount of medication absorption was measured using the skin of a pig ear that was procured from a local butcher. The donor compartment's phosphate buffer-containing receptor compartment was covered with invasomal formulation, which was then filled with it (pH 7.4). Pig ear skin was sandwiched between the two compartments and secured with a clamp. At a temperature of 37°C with constant magnetic stirring, the experiment was run for 24 hours. Samples from the receptor cell were collected at premediated time points of 1, 2, 3, 4, 5, 6, 8, and 10 h, and their AG concentrations were determined using a UV spectrophotometer set at 224 nm. The results of the ex vivo experiment were used to compute the skin parameters flux (J, g/cm2/h), cumulative permeation of AG over the skin per unit area (Q24, g/cm2), permeability coefficient (Kp, cm/h), and enhancement ratio (ER) [21].

Skin retention studies

To evaluate how much AG was retained in the skin after the ex-vivo permeation study, the pig ear skin was separated, vortexed in methanol (10 ml) for 10 min, and then kept in

methanol overnight. The concentration of AG in the solvent methanol was calculated using a UV spectrophotometer with a 224nm setting[22].

Morphology and vesicular shape

The ideal invasomal formulation was observed using a transmission electron microscope (TEM) (Technai) with an accelerating voltage of 100 kV. The samples were negatively stained with a 1% phosphotungstic acid aqueous solution. Before staining, the invasomal mixture was dried on a tiny carbon-coated grid. By blotting, surplus solution was taken out. The substance was examined under a microscope after drying[23].

Preparation of AG invasomal gel

The carbopol 934 gel basis was supplemented with an invasive formulation that had the largest cumulative AG release, smallest particle size, and highest ex vivo permeability. To make a 1% carbopol gel, the invasomal dispersion was slowly added to the gel foundation and moderately stirred using a mechanical mixer[24, 25].

Assessing AG invasomal gel

Physical assessment

By using visual inspection, the gel's physical features, washability, occlusiveness, clarity, and organoleptic characteristics were investigated.

Drug content studies and pH

500 mg of the invasomal gel sample were taken from 3 different points to guarantee AG consistency throughout the gel. Each sample was extracted in 10 ml of methanol, followed by 15 minutes of centrifugation at 3000 rpm. The samples' supernatants were collected, and the concentration of AG was found[26]. The pH of the AG invasomal gel was measured employing a pH metre. Measurements were made three times, and the standard value was calculated[27].

Homogeneity and grittiness

Pressing a tiny bit of gel among the index finger and thumb allowed researchers to gauge how gritty the ethosomal gel was. To determine the consistency of the gel, it was carefully examined for any attendance of coarse particles on the fingertips. By rubbing a little amount of the gel on the skin on the back of the hand, it was possible to determine the homogeneity of the gel being tested[27].

Spreadability and extrudability study

By calculating the alter in diameter when 500 mg of gel was put among two horizontal plates of 20×20 cm^2 gel and a standard weight of 125 g was placed over it, the spreadability of the invasomal gel was investigated. The weight in grammes wanted to make a 0.5 cm ribbon of gel in 10 seconds using the prepared invasomal gel was used to determine its extrudability [22].

In vitro drug release

The receiver cell volume and effective permeation area used for the in vitro drug release experiment were 10 ml and 0.196 cm^2, respectively, in Franz's diffusion cell. The donor cell containing the invasomal gel was clamped over by the receptor cell, which was filled with phosphate buffered saline (pH 7.4). The experiment was run continuously for 24 hours at 37°C with 600 rpm magnetic stirring. The amount of AG in samples obtained from the receptor cell at specific times1, 2, 3, 4, 5, 6, and 24h was determined using a UV spectrophotometer set at 224 nm. To ascertain the release kinetics of invasomal gel, the data was processed using a variety of release kinetics models[19, 20].

Study of the invasomal gel's permeation (ex-vivo studies)

An ex vivo drug permeation research was performed using a Franz diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^2, respectively. The amount of medication absorption was measured using the skin of a pig ear that was procured from a local butcher. The donor compartment was filled with invasive gel, and the phosphate buffer-containing receptor compartment was covered with it (pH 7.4). Pig ear skin was sandwiched between the two compartments and secured with a clamp. The experiment was run continuously with magnetic stirring for 24 hours at 37±1°C. Using a UV spectrophotometer set at 224 nm, samples that were taken from the receptor cell at certain intervals1, 2, 3, 4, 5, 6, 8, and 10 hours were calculated for their AG concentration. Using data from the ex vivo experiment, skin characteristics such as flux (J, g/cm2/h), cumulative permeation of AG across the skin per unit area (Q24, g/cm2), permeability coefficient (Kp, cm/h), and enhancement ratio (ER) were determined[20].

Stability studies

The invasomal gel was maintained at 4-8°C and 27-30°C for a month. Each week, the samples' physical characteristics, pH, and medication content were assessed[28].

Results and discussion

The polydispersity index for all the invasomal formulations was determined to be less than 0.34, and the vesicle size of the formulated AG invasomes assortment from 292.05± 2.44 to 334.66±1.04nm. The terpene content rose along with the particle size. The invasomes' particle size is increased by the lipophilic terpene that is loaded with a high concentration of lipophilic medication, is responsible for the observed vesicle size of 334.66±1.04nm at 1.5% concentration of nerolidol. The produced invasomes' PDI values demonstrate superior stability and uniformity of the vesicles (Figure 1). The amount of drug trapped in the vesicles straight indicates how well the drug can adapt to the lipid content and form vesicles that are highly stable. The range of the AG EE in various invasomal formulations was 76.08±0.49 to 88.65±0.48% (Figure 2). The findings imply that an increase in terpene content improved the solubilization of the lipophilic medication, which in turn improved the EE of the manufactured invasomes. When vesicles are formed, the vesicular bilayer, where the phospholipid acyl chains offer a suitable environment for the lipophilic medication and lipophilic terpene, is where the lipophilic terpene dissolves with phosphatidylcholine. This confirms that additional chains become accessible as terpene concentration rises, improving the lipophilic drug's solubility in bilayers and, consequently, entrapment efficiency[29]. Transmission electron microscopy (TEM) analysis of the vesicle morphology indicated that round spherical unilamellar vesicles with an even surface predominated (Figure 3). The particle size estimated by TEM and the particle size estimated by dynamic light scattering were in agreement (Table 2). The total quantity of AG discharged from the invasomal formulations was contrast to the quantity released from a straightforward AG solution. After 24 hours, the cumulative releases for the invasomal formulations N1, N2, N3, and N4 were 3562.56 ±4.62, 4125.14 ±2.45, 4425.45 ±2.86, and 4652.19± 1.54 g/cm^2, respectively, while the release for the AG solution was 1558.50±4.21 g/cm^2 (Figure 4). The concentration of terpene was thought to have a significant impact on the drug release profile because N4 demonstrated the highest quantity of drug release. With an enhance in terpene content, more AG was released. Several release kinetics models, including first order, zero order, Higuchi's

equation, and Korsmeyer Peppa's equation, were used to analyze the in vitro drug release data. The model with the highest correlation (R^2) coefficient value was discovered to be the most effective at characterizing the drug release kinetics of the improved invasomes. The best correlation coefficient value for the Higuchi model was shown by the optimized formulation (N4), which was followed by the Korsmeyer-Peppas (R^2=0.933), first-order (R^2=0.876), and zero order (R^2=0.845) models. It was found that the optimized invasomal formulation (N4) corresponded well to Higuchi's model kinetics, which describes release of the drug as a time-dependent diffusion process based on Fick's law, after analyzing the in vitro drug release data using the pertinent model equations. The Korsmeyer-Peppas model was further employed to better define the drug-release behavior. According to the release exponent (n>1) of the Korsmeyer-Peppas equation, the release of AG from N4 is consistent with case II of the transport mechanism, in which a drug's release is controlled by polymer erosion and relaxation. A modified Franz diffusion cell was used for an ex-vivo drug permeation research, and the total amount of AG that permeated from the invasomal formulations was compared to AG solution. After 24 hours, the cumulative permeability for the invasomal formulations N1, N2, N3, and N4 was 3248.34±3.62, 3582.14 ±3.45, 3859.25± 3.86, and 4056.19±1.54 g/cm^2, respectively, whereas the penetration for the AG solution was 956.40± 5.21 g/cm^2 (Figure 5). The concentration of terpene had a significant impact on the drug permeation profile, with N4 exhibiting the highest level of drug penetration. With an increase in terpene concentration, more AG penetrated the stratum corneum layer of the skin. Table 3 shows the findings from an analysis of the cumulative quantity of AG that permeated after 24 hours, flow, enhancement ratio, permeability coefficient, and skin retention. For AG invasomes, the permeation flux value was reported to range from 171.56±0.12 to 195.58± 0.54 g/cm^2/h, whereas plain AG solution showed a permeation flux of 68.55 ±0.03 g/cm^2/h (Figure 6). When compared to AG solution, the permeation flux increased by 2.6 times in invadesomes containing 1.5% nerolidol. It was discovered that the invasomal formulation's permeability coefficient ranged from 17.2×10^{-3} to 19.2×10^{-3}cm/h. The permeability coefficient of the AG solution was 6.4 $\times10^{-3}$cm/h, but the permeability coefficient of the N4 invasomal formulation was 2.5 times greater. The pig ear skin contained between 436.72 ± 0.31to 756 ±0.25µg/cm^{2}of AG that was maintained. When compared to the AG solution, which had drug retention of 228.56±0.89µg/cm^2, the invasive formulation N4 had the maximum retention at 756±0.25µg/cm^2. The larger invasomal vesicle is thought to be the cause of the higher amount of AG retained in the skin along with an increase in terpene concentration. N4 was added to the gel foundation to create an invasive formulation with high cumulative curcumin release, good entrapment efficiency, small particle size, and strong ex vivo penetration (carbopol 934). The invasomal gel had an appearance of white to off white, was easily washing, was free of any gritty particles, and had high homogeneity. The pH of the gel formulation was 6.2±0.05, which falls within the normal pH range for skin [22]. The produced gel showed an occlusive quality that increases the penetration of the active ingredient, increasing the efficacy. The composition of the invasive gel revealed a consistent gel with 98.72± 0.013% AG concentration. Spreadability of a gel is particularly important for patient compliance since it makes it easier to apply gel evenly to the skin surface. A good gel is regarded as having a higher spreadability and requiring less time to spread. When 125 g of weight were added to the plates, the gel's spreadability rose by 4.9 times, indicating that the

invasomal gel has a good spreadability (Table 4). When clotrimazole vaginal gel based on microemulsion was created, similar outcomes were found. Their findings demonstrated that the spreading diameter grew in response to the weight's effect[30]. In another study, the spreadability of curcumin ethosomal gel increased five times once weight was added. During application, gel extrusion from the packed tube is crucial. Remarkable consistency gels are difficult to extrude, whereas gels with low viscosity exhibit high fluidity. As a result, a gel with the right consistency is necessary for optimum extrudability. The AG invasomal gel was able to extrude a 0.5 cm ribbon of gel in about 10 seconds, indicating that the gel has good extrudability. Nano-invasomal gel of olmesartan medoxomil designed for transdermal administration showed similar extrudability results[11]. The cumulative amount of AG released from the invasomal gel was compared to the amount released from a straightforward AG gel in *in vitro* drug release tests using modified Franz diffusion cells. After 24 hours, the cumulative release from the invasomal gel was 4552.19±13.11µg/cm^2, whereas the release from the AG gel was 1574.5±16.12µg/cm^2 (Figure 7). The findings suggested that, when compared to plain AG gel, the invasomal gel has an excellent release profile. Several release kinetics models, including first order, zero order, Higuchi's equation, and Korsmeyer Peppa's equation, were used to analyze the in vitro drug release data. The model with the highest correlation (R^2) value was discovered to be the most effective one for explaining the drug release kinetics of the invasomal gel. The gel exhibited good conformity to the Higuchi model (R^2=0.933), followed by the Korsmeyer-Peppas (R^2 = 0.911), first-order (R^2 = 0.856), and zero order (R^2 = 0.845) models. The Korsmeyer-Peppas model was further employed to better define the drug-release behavior. The Korsmeyer-Peppas equation's release exponent (n>1) suggests that the AG release from nano vesicular gel conforms to case II transport mechanism, in which the polymer's erosion and relaxation control how much drug is released. The cumulative amount of AG that permeated the skin from the invasomal gel was compared to the amount permeated from a standard AG gel in an ex-vivo drug permeation investigation using a modified Franz diffusion cell. After 24 hours, the invasomal gel displayed a cumulative permeation of 3954±10.32g/cm^2, whereas the penetration of the standard AG gel was 1068±10.02g/cm^2 (Figure 8). The synergistic action of ethanol and terpenes is responsible for the enhanced penetration of AG over the skin from the invasomal gel. Alcohol renders the vesicles malleable, and terpene fluidizes the stratum corneum's lipid layer, disrupting the lipid's normal arrangement and making it easier for invasomal vesicles to penetrate the stratum corneum's lipid layer [31]. According to the results, the permeation flux of AG from invasomal gel and simple gel were determined to be 155.02± 0.12 and 61.56± 0.17g/cm^2/h, respectively. This indicated that the permeation flux of AG from invasomal gel was 2.5 times greater than simple AG gel (Table 5).

Table 2: Particle size, PDI and entrapment efficiency of AG invasomes

Formulation code	Vesicle size (nm) ± SD	PDI ± SD	Entrapment efficiency (%) ± SD
N1	292.05 ± 2.44	0.34± 0.008	76.08± 0.49
N2	304.18± 1.44	0.32± 0.005	82.15± 0.44
N3	316.33 ± 2.15	0.31 ± 0.003	85.26± 0.77

N4	334.66 ± 1.04	0.26± 0.004	88.65± 0.48

TABLE 3: Skin permeation parameters of AG invasomes across the pig ear skin

Formulation code	Q_{24} Cumulative drug(µg/cm^2) ± SD	Permeation flux (J, µg/cm2 /h) ± SD	Permeability coefficient (Kp, cm/h) ± SD	Enhancement Ration (ER)	Amount deposited (µg/cm2) ± SD
N1	3248.34 ± 3.62	171.56 ± 0.12	17.2× 10^{-3}	2.54	436.72 ± 0.31
N2	3582.14 ± 3.45	178.25 ± 0.96	17.8 × 10^{-3}	2.65	529 ± 0.56
N3	3859.25 ± 3.86	190.74 ± 0.85	18.6 × 10^{-3}	2.74	623 ± 0.89
N4	4056.19 ± 1.54	195.58 ± 0.54	19.2 × 10^{-3}	2.83	756 ±0.25
Drug	956.40 ± 5.21	68.55 ± 0.03	6.4 × 10^{-3}	1.00	228.56 ± 0.89

TABLE 4: Spreadability of gel

	Diameter (cm)	Area (cm^2)
Before (adding weight)	1.3cm	1.43
After (adding weight)	3.6cm	6.45

TABLE 5: Skin permeation parameters of ag invasomal gel and simple AG gel across the pig ear skin

Formulation Code	Q24 Cumulative drug permeated (µg/cm^2) ± SD	Permeation flux (J, µg/ cm^2 /h) ± SD	Permeability coefficient (Kp, cm/h) ± SD	Enhancement Ration (ER)	Amount deposited (µg/cm^2) ± SD
Simple AG gel	1311.47 ± 10.02	61.56 ± 0.17	6.2 × 10^{-3}	1	245 ± 0.07
AG Invasomal Gel	3789.55 ± 9.33	155.02 ± 0.12	156× 10^{-3}	2.501	656.36 ± 0.36

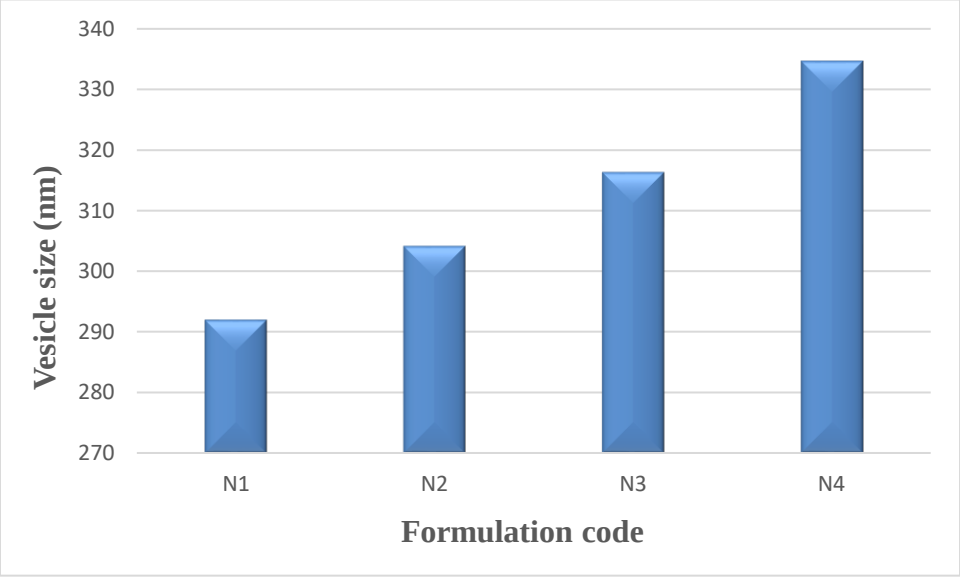

Fig. 1: Vesicle size of AG invasomes

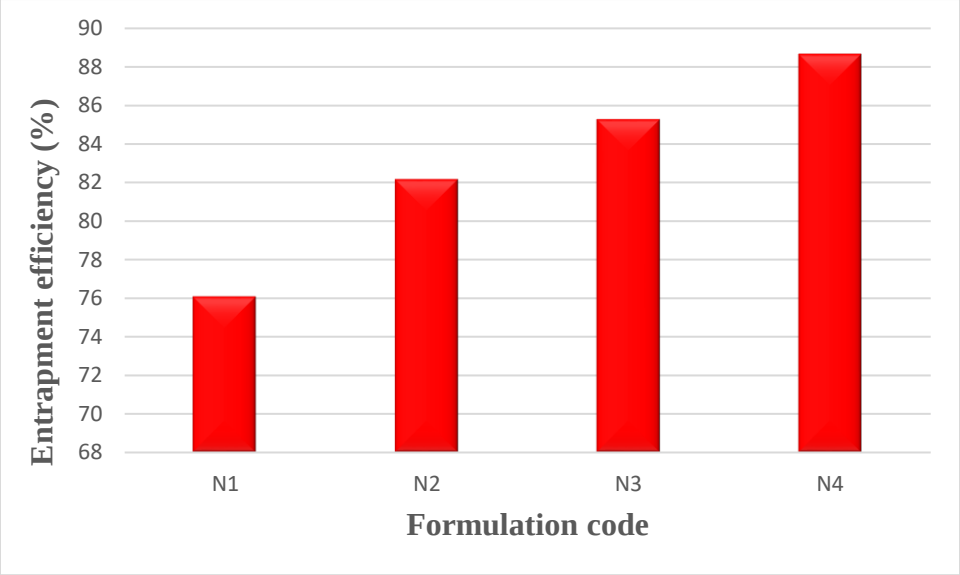

Fig. 2: Entrapment efficiency (%) of AG invasomes

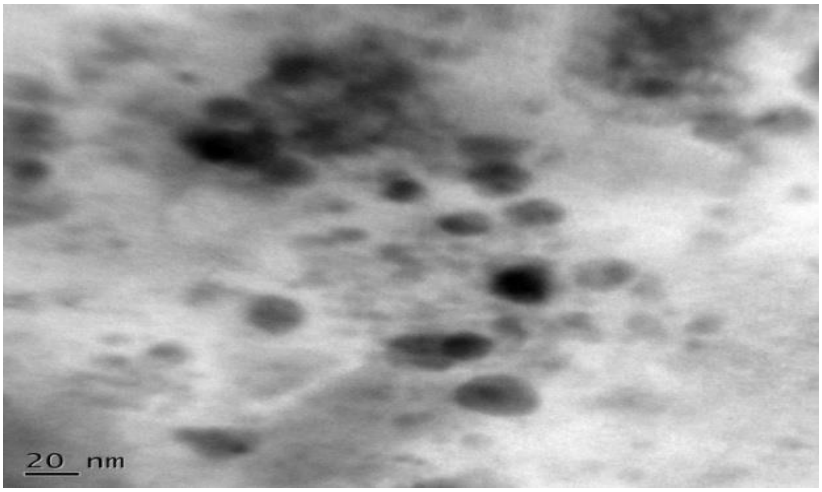

Fig. 3: TEM image of invasome

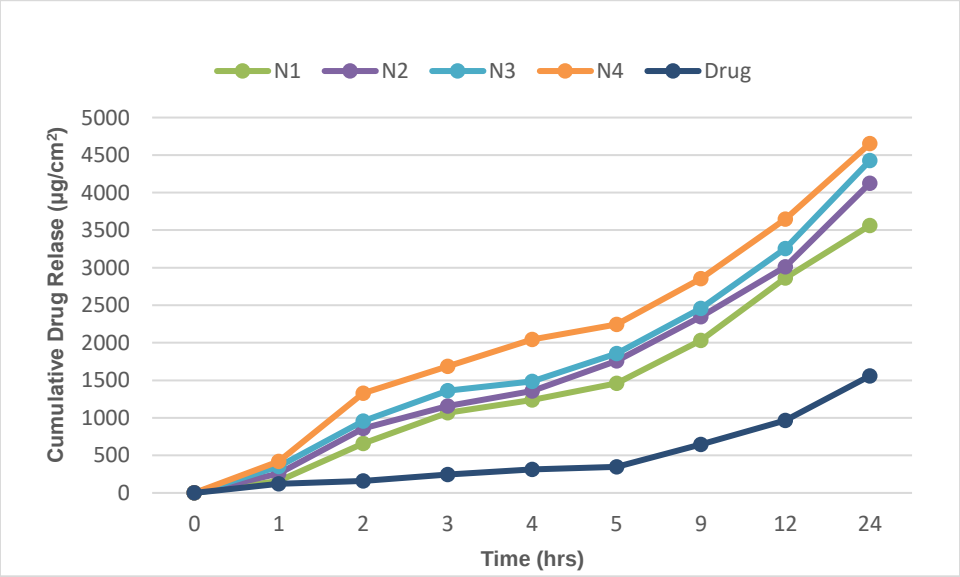

Fig. 4: Cumulative amount of AG released (µg/cm^2) from invasomes and AG solution

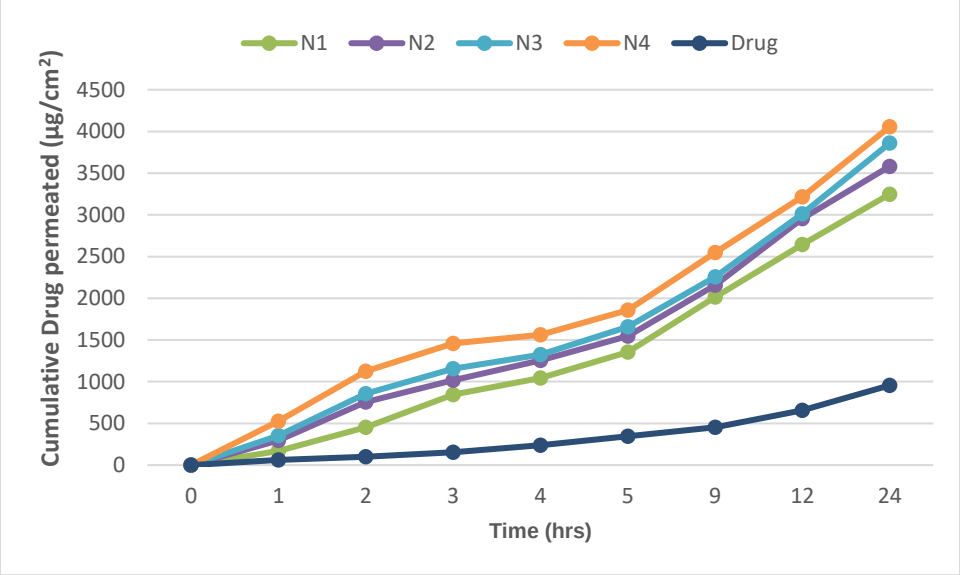

Fig. 5: Cumulative amount of AG permeated (µg/cm^2) from invasomes and AG solution

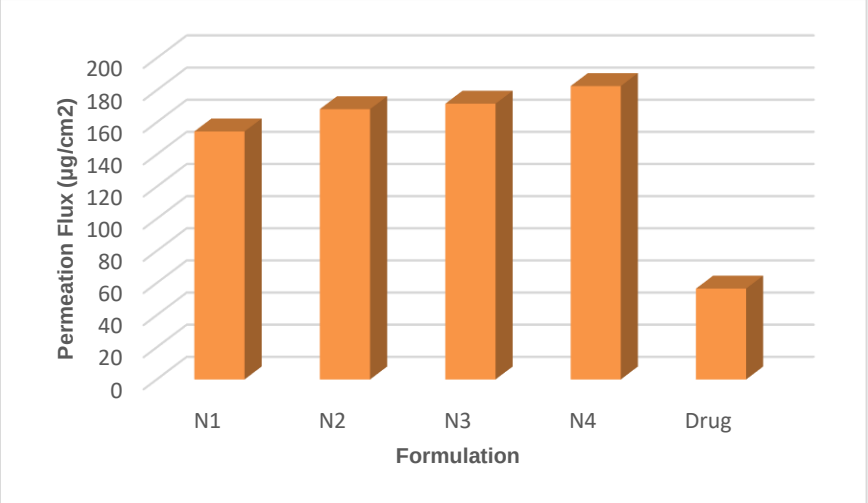

Fig. 6: Comparison of permeation flux of AG invasomes and simple solution

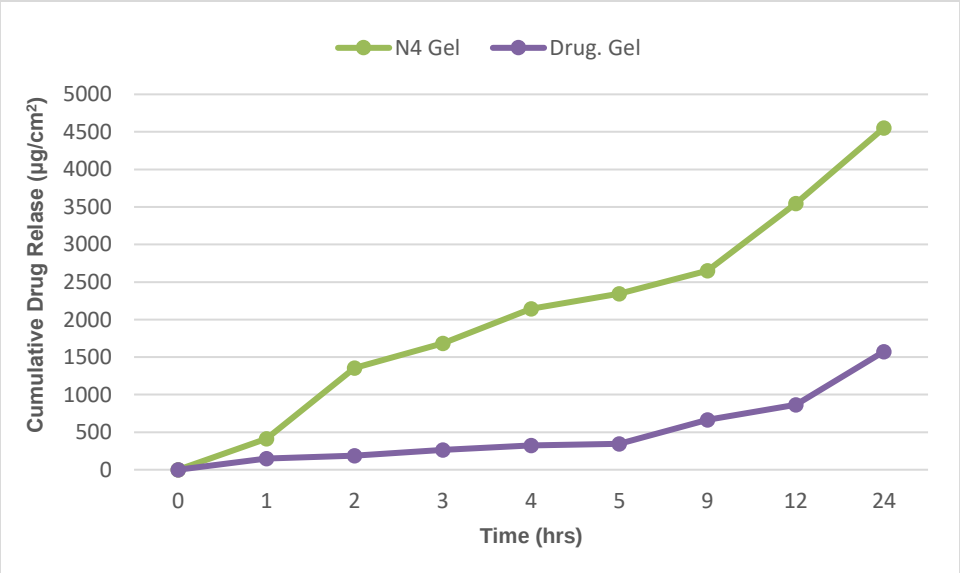

Fig. 7: Cumulative amount of AG released (µg/cm^2) from invasomal gel and AG gel

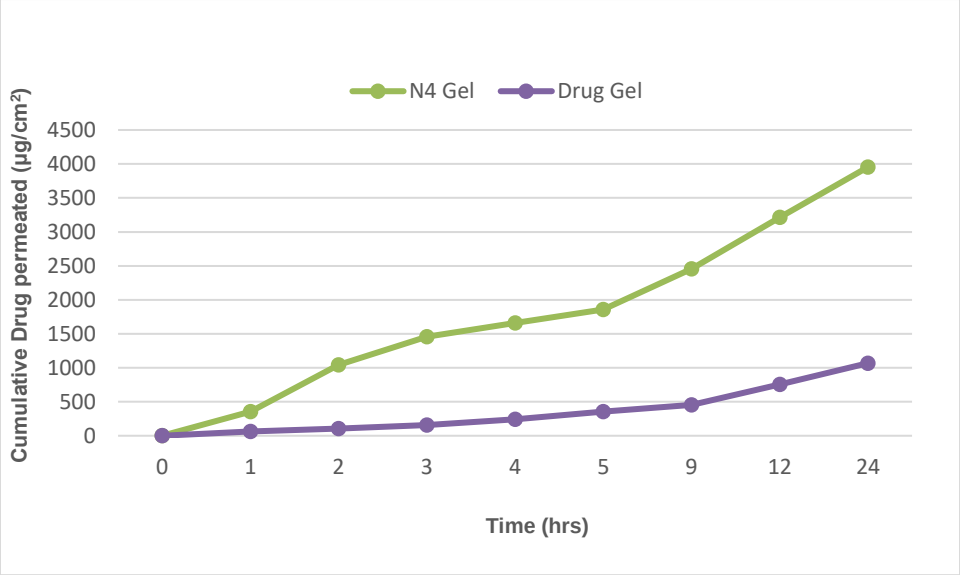

Fig. 8: Cumulative amount of AG permeated (µg/cm2) from invasomal gel and AG gel

Conclusion

Transdermal andrographolide delivery employing invasomes as a potential carrier was studied. Nerolidol was used as a permeation enhancer when mechanical dispersion was used to create andrographolide-loaded invasomes. The generated invasomal formulations underwent in vitro drug release, EE, vesicle size, skin retention, and ex vivo permeation investigations before being characterised and optimized. The greatest amount of drug entrapment, as determined by the ultracentrifugation method, was found to be 88.65 ±0.48%. The produced vesicles' high stability and homogeneity were confirmed by the polydispersity index, which was determined to be less than 0.34 and ranged in size from 292.05±2.44 to 334.66±1.04nm. Under a transmission electron microscope, the improved invasomal formulation revealed spherical-shaped vesicles with a vesicle size of 334.66± 1.04nm. The improved invasomal formulation was combined with carbopol 934 bases to create a topical gel that was white to off white in colour, had a pH of 6.2±0.05, and contained 98.72±0.013% active andrographolide. It also demonstrated good extrudability, spreadability, and homogeneity. According to an ex vivo study, the invasomal gel's andrographolide penetration across the skin of the pig ear was 2.50 times greater than that of regular andrographolide gel, with a flow of 155.02±0.12 g/cm2/h. When compared to plain andrographolide gel, the invasomal gel was able to achieve a higher flux and maximum skin retention because of the synergistic effects of nerolidol and ethanol, which disrupt the lipids of the stratum corneum, work with intercellular protein molecules, and intensify the partitioning of andrographolide into the skin layer. The study's findings indicate that invasomes have a good chance of delivering andrographolide transdermally, and invasomal gel can increase patient compliance while also being effective in managing and treating a variety of skin conditions because the medication is offered in a topical gel that is easily applied to the skin and is available in a semisolid form. Terpenes and ethanol are present in invasomes, which distinguish them from one another. When compared to other micro vesicles like liposomes and ethosomes, they offer a high level of membrane fluidity, deformability, and penetration, which is attributed to the synergistic effect of ethanol and terpene. These vesicles have demonstrated the

synergistic benefits of liposomes, which can change the packing hierarchy of the stratum corneum to enhance skin delivery.

Author's declaration of consent

According to the signature, the text is unique, has never been published, and is not currently being considered for publishing anywhere. We confirm that the paper has been read, reviewed, and approved by all of the indicated authors and that no other people who are eligible to be an author but are not listed have worked on the project. We further reiterate that the order in which the authors are listed in the manuscript is one that we all agreed upon. We realize that the corresponding author is the only person with whom the editorial process can be in contact. He or she is in charge of informing the other authors about the status of the work, the submission of amendments, and the final approval of proofs.

Statement of conflicting interest

No conflicts of interest

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NA

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