FORMULATION DEVELOPMENT AND CHARACTERIZATION OF *NIGELLA SATIVA* LINN LOADED ETHOSOMES AND TRANSETHOSOMES GEL FOR THE TREATMENT OF PSORIASIS: A COMPARATIVE ASSESSMENT

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Abstract

Herbal compounds have different physicochemical properties. Its use on the oral route often has low biological availability. Therefore, alternative transdermal routes are used through the skin. The stratum corneum skin layer is the most difficult layer to penetrate. Therefore it is necessary to use a drug delivery system such as ethosomes, transfersome or transethosomes to increase transdermal drug delivery. Ultradeformable vesicles (UDV) have recently become a promising tool for the development of improved and innovative dermal and transdermal therapies. The aim of this investigation was to formulate, evaluate and compare the transdermal potential and psoriasis activity of novel vesicular carriers: transethosomes and ethosomes of *Nigella sativa*. Transethosomes (Te) and ethosomes (E) were prepared by thin-layer hydration and cold method respectively and were characterized for zeta potential (ZP), vesicle size and polydispersity index, shape and surface morphology and entrapment efficiency. Optimized Te and E formulation was incorporated into gels was characterized by viscosity, pH measurement, spreading diameter, *in vitro* release studies, permeation data analysis, drug content, fluorescence microscopy.Transethosomal and ethosomal formulation showed particle size of 172±11nm and188±11nm. Transethosomes showed drug entrapment (71.7±2.7%) than ethosomes (78.6±3.1%). Both the formulation showed good zeta potential indicating good stability.Drug content of transethosomal and ethosomal gel formulation was found to be 74.1±1.9 and 70.5±2.1 respectively. *In vitro* drug release through animal skin of transethosomes and ethosomes gel was found to be 98.23% and 95.65% respectively. The prepared transethosomes gel of *N. Sativa* extract loaded was found to be more effective in imiquimod-induced psoriasis; DNCB induced dermatitis and in inflammation as compared to *Nigella sativa* extract loaded ethosomal gel. This may be due to the fact that incorporation of phytoconstituents into vesicular system like transethosomes resulted in much better absorption profile which enables them to cross the biological membrane, resulting in enhanced bioavailability. The therapeutic action becomes enhanced, more detectable, and prolonged. Hence, the results suggested transethosomes to be a more efficient carrier system as compared to ethosomes for transdermal delivery of *Nigella sativa.*

Keywords: *Nigella sativa* Linn, Transethosomes, Ethosomes, Psoriasis activity, Ultradeformable vesicles, Thin-layer hydration

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Introduction

The skin is the outer organ, whose main role is protecting our body from external insults, acting as a barrier against environmental stressors and injuries [1]. Among the three layers composing the skin epidermis, dermis and hypodermis the major barrier function is played by the epidermis, with its outer layer constituting non-viable cells (i.e., stratum corneum) and the inner layers made of viable keratinocytes (i.e., stratum lucidum, stratum granulosum, stratum spinosum and stratum basale). On the other hand, the dermis is a greatly vascularized layer, making the skin a significant drug administration route. In particular, the topical route may be the best option to treat skin conditions and disorders [1,2].Nevertheless, due to its peculiar structure, the stratum corneum layer acts as a barrier, hindering the passage of drugs through the skin [1]. To overcome this problem, many research efforts have been carried out to design efficacious transdermal delivery systems [3].Particularly, colloidal-based delivery systems made of biocompatible and biodegradable phospholipids were developed. The former approach was based on the use of liposomes, bi-layered vesicles usually constituted of phospholipids, such as phosphatidylcholine (PC),and water, which is able to deliver both hydrophilic and hydrophobic molecules [3-5].However, liposomes proved to not be flexible enough to penetrate the skin, forming adepot in the stratum corneum, from which the encapsulated drugs could slowly diffuse [6].Therefore, deformable liposomes were developed using edge activators (e.g., surfactants)in order to destabilize the lipid bilayers formed by PC and water, possibly improving the flexibility of the vesicles [7,8]. More recently, a significant improvement of vesicle flexibility was achieved by Touitou's invention of ethosomes (E) [9-11]. Ethosomes composition is based on phospholipids (such as PC), water and an ethanol content comprised between 20 and 45% v/v. Ethosomes can solubilized both lipophilic and hydrophilic drugs inside the vesicles. The presence of ethanol confers elasticity to the vesicles, due to its ability to interact with both skin and vesicle lipids, promoting the passage of the entrapped drug through the stratum corneum. Moreover, ethanol improves the vesicle thermodynamic stability and loading capacity of lipophilic drugs in comparison to liposomes [11-13]. To further increase the vesicle penetration ability, transethosomes (TE) have been developed. TE is composed of phospholipids, such as PC, water, ethanol and edge activators, such as surfactants [13-15]. TE should therefore possess the advantages of both deformable liposome and E, being constituted of edge activators and ethanol, as well as PC and water [15]. Therefore, E and TE can be described as flexible PC-based nano-vesicular systems that are suitable for achieving the transdermal delivery of many drugs, offering the chance to treat several skin pathologies [16]. Psoriasis a skin disorder characterized by unraced cutaneous, demolition, agitated proliferation and poor isolation of epidermal keratinocytes. It is a recurrent, chronic inflammatory problem, and is associated with severe morbidity and social stigma making it an impending threat [17-19]. The prevalance of psoriasis worldwide ranges between 0 and 6.6%, where as in India, it has been recently reported as high as 8% [20, 21]. A broad range of treatment options involving systemic therapy [22], topical therapy [23], and phototherapy [24], are available and practiced. Amid these, the topical therapy is generally preferred owing to the ease of application, localized impact and reduced systemic load [25], esp. in the cases when the body surface area covered is not more than 50%. *Nigella sativa* (NS) which is an annual flowering plant, belonging to family Ranunculaceae, has been used for centuries for the treatment of many skin disorders including psoriasis. Much of the anti-psoriatic activity of NS seeds has been attributed to the presence of thymoquinone (TQ), which is one of the major component of essential oil but also present in the fixed oil. Topical administration of TQ, which is a lipid soluble benzoquinone, has attracted special attention, as it exerts the therapeutic action against several skin pathologies, viz. acne vulgaris, skin pigmentation, vitiligo, hypersensitivity reactions, early stages of skin tumor genesis including psoriasis [26]. TQ exerts its effect by acting as a free radical and superoxide radical scavenger, mean while preserving the activity of various anti-oxidant enzymes such as catalase, glutathione peroxidase and glutathione-S-transferase [27]. Despite of having promising benefits for skin therapeutics, complete clinical utilization of TQ has been limited owing to its high hydrophobicity, poor aqueous solubility, poor bioavailability, chemical instability, and its poor penetration through the system [28]. Therefore, an effective delivery is needed to increase the solubility, protect the drug and at the same time facilitate penetration and retention in the hyperkeratotic skin of psoriatic lesions. So, the aim of this investigation was to formulate, evaluate and compare the transdermal potential and psoriasis activity of novel vesicular

carriers: transethosomes and ethosomes of *Nigella sativa*.

Materials and methods

Plant material

The seeds of *Nigella Sativa* were collected from the local market of Solan, Himachal Pradesh. The plant was authenticated by Dr. N.K. Dubey, Professor, Centre of Advanced Study in Botany Institute of Science, Banaras Hindu, University, Varanasi, U.P. and Voucher specimen No. Acantha. 2021/1was deposited in department. The plant seeds were dried, powdered coarsely, passed through 40 No. sieves and lastly stored in an airtight container.

Chemical reagents

Lipoid P30, carbopol 934, polyethylene glycol was purchased from Sigma-Aldrich Chem, Germany. High purity 99.9% ethanol was obtained from SD Fine chemicals, Mumbai, India. Soya phosphatidyl choline (SPC), span 80, phospholipid 90G was purchased from SD Fine-Chem Ltd. Mumbai, India. All other chemical and materials were of analytical grade. Triple distilled water was generated in house.

Extraction of plant material

The extraction of *Nigella sativa* seed was done using 10 gm seed powder with ethanol (0-100%), using different extraction time (60-120 min) and temperature (40-60 ºC) in different solid to the solvent ratio (1:10-1:20). The study was optimized for the extraction of phenolic content and removed the solvent using vacuum and stored at -22ºC for further study [29].

Preparation of *N. sativa* seeds ethanol extract loaded transethosomes

Transethosomes was prepared using the thin-layer hydration method. Dissolved span 80 and lipoid P30 in dichloromethane and removed the dichloromethane by rotary evaporator at 40°C. Pass the nitrogen gas to make a thin layer and stored the reaction vessel in the refrigerator for 24 hours. Rehydrated the prepare thin layer using ethanol *N. sativa* seeds extract in 95% ethanol (7 ml) and further add phosphate buffer (pH 5.5; 18 ml). Shake the reaction vessel by rotation for 1 h at 37°C and stored the prepared transethosomes in the refrigerator at 4°C.

Optimization of lipid P30 concentration

To optimize lipoid P30 concentration, prepared vesicles using lipoid P30 concentration at three different concentrations with optimized extract concentration. The optimized formulation showed highest entrapment efficiency.

Optimization span 80 concentration

The span 80 concentration was optimized using three different concentrations with optimized extract concentration and lecithin concentration. The optimized formulation showed highest entrapment efficiency.

Table 1: Optimization of Lipid P30 and Span 80 concentration

Formulation Code	Lipid P30 (mg)	Span 80 (mg)
Te1	4.0	0.50
Te2	4.0	0.75
Te3	4.0	1.00
Te4	5.0	0.50
Te5	5.0	0.75
Te6	5.0	1.00
Te7	6.0	0.50
Te8	6.0	0.75
Te9	6.0	1.00

Preparation of *N. sativa* seeds ethanol extract loaded ethosomes

N. sativa seeds ethanol extract loaded ethosomes were prepared using cold method with varying concentrations of Soya phosphatidyl choline (1-3%) and ethanol (10-40%). Soya phosphatidyl choline [Table 2] was dissolved in ethanol at 30ºC, adds 10 mg of *N. sativa* seeds ethanol extract slowly using a syringe and stirred for 15 min at 900 rpm. Sonicate the formulations for 5 min and refrigerated [30].

Optimization of ethanol: distilled water ratio

The ethanol: distilled water ratio was optimized at four different concentrations with optimized extract concentration. The highest entrapped vesicles were selected.

Optimization lecithin concentration

To optimize lecithin concentration vesicles were prepared at three different levels using pre-optimized extract concentration and ethanol: distilled water ratio. The highest entrapped vesicles were selected.

Table 2: Formulation table of various *N. sativa* seeds ethanol extract loaded ethosomal batches

Formulation Code	% SPC	Ethanol: Water
E1	1%	20:80
E2	1%	30:70
E3	1%	40:60
E4	2%	20:80
E5	2%	30:70
E6	2%	40:60
E7	3%	20:80
E8	3%	30:70
E9	3%	40:60

Characterization of *N. sativa* seeds ethanol extract loaded vesicular systems

Furthermore, all the prepared nano formulations were characterized with the help of zeta potential (ZP), vesicle size, polydispersity index, shape and surface morphology, entrapment efficiency[31]. Prepared vesicles were visualized by phase contrast microscope at 100X and take the photographs using Olympus camera (Olympus MJU 9010).

Zeta potential (ZP), vesicle size and poly-dispersity index

Zetasizer (Malvern Instruments, Malvern, Ver 7.03) was used to determine vesicle size, Zeta potential (ZP) and polydispersity index (PDI).

Shape and surface morphology

These parameters of nanovesicles were determined by transmission electron microscope (Jeol® JEM 2100, USA). The nano-formulations (50 µl) were diluted and 50 µl of solution was applied and visualized using microscope with 10-30 kV voltage.

Entrapment efficiency

Aliquots of nano-vesicular formulations were centrifugation at 12000 rpm via cooling centrifuge (Remi). The supernatant was removed separate the unentrapped extract and added 1ml of 0.1% Triton X 100 in sediment to lyses the vesicles and further diluted with PBS (pH 7.4). Determined the percentage of flavonoid content in terms of quercetin. Quercetin is present in *N. sativa* in terms of flavonoid, responsible for its pharmacological property, and thus considered as a biomarker for estimations [32]. The %EE was calculated using the formula:

% EE = (amount of chemical constitute in sediment ÷ chemical constitute in the extract added to nano-vesicular formulations) ×100

Development of hydrophilic gel formulation

Among the nano-vesicular formulations, optimized formulation has highest EE, optimum PDI and zeta potential were further utilized to incorporated into Carboxyvinyl polymer carbomer (Carbopol 934P) gel formulations. All two optimized formulations i.e. transethosomes (Te3) and ethosomes (E7) were separately utilized for gel formulation (i.e. GELTe3 and GELE7 respectively). Carbopol 934P (1.0% w/w) was soaked in water for an hour and add 10 ml of nano-vesicular dispersion containing *N. sativa* seeds ethanol extract (10 mg). Stirred the mixture at 800 rpm at 30ºC to make homogeneous gel. Maintain the neutral pH of prepared gel by triethanolamine [33].

Characterization of hydrophilic gel formulation

Viscosity and pH measurement

The viscosity of ethosomal gel formulation was measured using Brookfield viscometer (Model No DV-III ULTRA) using spindle no 06 at 100 rpm and pH measurements of the formulations were done using digital pH meter (RI-152-R).

Spreading diameter

The spread ability of gel formulation was determined by measuring the spreading diameter of 1 gm of gel between two horizontal plates (20 cm x 20 cm) after 1 min. The standard weight applied on the upper plate was 125 gm.

***In vitro* release studies**

In vitro diffusion study of all two gel formulations were determined and compared with optimized nano-vesicular formulations and conventional gel formulation using dialysis membrane (Hi-media). The gel was placed in PBS (7.4) for 4 hrs and then mounted between the donor and receptor compartment of the Franz diffusion cell (surface area for diffusion was 2.54 cm^2). To perform ex vivo permeation study using animal skin full-thickness goat ear skin (procured from local abattoir; used within 24 h) was used. The skin was mounted in between receptor and donor compartments with the stratum corneum side facing upward into the donor compartment. The release rate of *N. sativa* was analyzed by placing the required sample in the donor cell compartment. To prevent contamination and evaporation, the donor compartment was covered with parafilm. The receptor chamber was filled with phosphate buffer PBS (7.4) and was maintained at 37ºC with continuous stirring. 1ml aliquot of receptor phase solution was withdrawn at time intervals of 0.5, 1, 2, 4, 8 and 24 hr and the same volume of fresh medium was added back into the chamber. The quantification was done using UV spectrophotometer (Shimadzu Model No. 1800) at 203 nm. The cumulative amount of drug permeated per unit area versus time graph was plotted and transdermal flux (J) was calculated from the slope of linear portion [34].

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 vs. time,

first order as the log cumulative percentage of drug remaining vs. Time, Higuchi's model as the cumulative percent drug remaining vs. square root of time, Hixson Crowell cube root model and Korsmeyer- Peppas model as the log cumulative percentage of drug released vs. log time.

Drug content of the formed gels

500 mg of the gel was taken and dissolved in 50 ml of pH 7.4 phosphate buffer (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 against corresponding gel concentration.

Fluorescence microscopy

The ability of fluorescence marker- loaded ethosomes 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 added in the ethanol along with SPC. Probe loaded vesicular dispersions were applied for 8 hrs to the dorsal skin of animal in Franz diffusion cell at 37^0C. At the end of experiment, skin was removed from the cell and thoroughly washed with distilled water. Treated area was cut out from the skin and sectioned using cryotome (Thermo scientific HM550). Skin specimens were observed under fluorescence microscope Leica DM 2500, using green filter N2.1, position 3 and irradiated with 540 nm and observed at a magnification of X 10.

In vivo anti-psoriatic activity

Experimental animals for psoriasis

Albino rats (Wistar strain) with no previous drug treatment were used for *in vivo* studies. Healthy albino rats (200-250gm) were received from the animal house of Madhyanchal Professional University, Bhopal (M.P.) India, placed in polypropylene cages and fed on a standard diet and water *ad libitum.* Acclimatization to laboratory hygienic condition was done for seven days before starting the experiment. Hairs on the dorsal portion of each rat were removed by using depilatory cream. The animal experimentation was performed according to institutional ethics committee granted permission under wide letter (Letter No. **PCP/IAEC/2023/JAN/13**).

Induction of Psoriasis

This method was a slight modification from the study design by van der Fits [35]. The animals were shaved on the dorsal skin. Imiquimod (IMQ cream Glenmark pharmaceuticals) 50 mg was applied topically on the shaven dorsal skin surface of each rat of Group 2- Group 5 to induce psoriasis once daily for 11 days [36].

Treatment of Psoriasis

Application of IMQ (50 mg) was continued throughout the treatment phase (for 12 days) to all the rats from G 1- G 5 groups, to ensure that the recovery, if any, in the ratis due to the test drug formulations. In the control group, animal's application of Vaseline was continued. Body weight, food and water intake of each animal were recorded daily.

Table 3: Groups and treatment for the *in vivo* anti-psoriatic activity

Group Number	Treatment
Group 1	Control (IMQ treated 50 mg)
Group 2	Positive control (Vaseline 50 mg)
Group 3	IMQ treated + Ethanolic *N. sativa* seed extract (amount~ 50 mg)
Group 4	IMQ treated + *N. sativa* seed extract loaded transethosomal gel (amount~ 50 mg)
Group 5	IMQ treated + *N. sativa* seed extract loaded ethosomal gel (amount~ 50 mg)
Group 6	IMQ treated + Conventional commercial formulation (Tazarotene 0.1% gel, Angle Gloss, Phytolab Pvt. Ltd.)

Animal Used: Albino rats (3 in each group); Condition: Controlled Temperature (25 ±2 º C), Relative Humidity (60 ± 5 %)

Histological studies

The dorsal skin was excised from the rat at the end of the experiment. The skin samples were immersed in 10 % formalin, embedded in paraffin wax, and sliced at a thickness of 3µm using microtome (Leica RM 2245). The samples were stained with hemoxylin and eosin (H and E) and observed under microscopy. The photographs of 40 X magnification (Leica DM 2500) of H & E stained skin sections of Group 1 to Group 7 animals were taken.

Anti- dermatitis study

Induction of dermatitis

Dinitrochlorobenzene (DNCB)-induced contact hypersensitivity (CHS) rat animal model is commonly used for studying the pathogenesis of dermatitis. 50µl 1%DNCB (Dinitrochlorobenzene) dissolve in acetone/olive oil (4:1) and apply

topically on outer and inner surfaces of right earlobe of each rat, 5 times at 48 hrs intervals to induce dermatitis, while the same quantity of acetone/olive oil (4:1) apply to the left side.

Treatment phase

N. Sativa extract loaded test formulations will be applied topically on the outer and inner surface of right ear lobes of animals daily for 7 days. Eight hrs after the final application induced ear swelling and mass will be measured and expressed as the increase from the pre-challenge value. Ear swelling changes (including ear mass and thickness) take place in the right ear lobes of DNCB induced dermatitis animal. Mass and thickness differences between left and right ear will be used to evaluate the induced ear swelling. Digital external micrometer (BAKER DMM25) will use to measure thickness. Then rats sacrifice and ears of both sides will be cut off using a hole punching device. Skin tissues from the right and left ear of mice will be excised and subjected to histological examination.

Results and discussions

Formulations of ethosomes and transethosomes having ethanolic seed extract of *N. sativa* were successfully prepared and characterized. Numerous formulation variables were optimized in all formulations on the basis of entrapment efficiency. Ethanolic *N. sativa* seed extract loaded transethosomes were successfully prepared by varying lipoid P30 and span 80 concentrations. Total nine formulations were prepared and evaluated for their entrapment efficiency, vesicle size, polydispersity index (PDI) and zeta potential. Formulation Te3 prepared with 4.0mg lipoid P30 and 1.0 mg span 80 having highest entrapment efficiency. Furthermore, all prepared formulation showed entrapment efficiency in the range between 53.7-71.7%, while vesicle size in the range of 167-199 nm, PDI in the range of 1.1-1.4 and zeta potential between -23.5 to -29.4. The optimized transethosomes formulation Te3 had entrapment efficiency (71.7±2.7%), vesicle size (172±11 nm), Polydispersity Index (1.3±0.3) and zeta potential (-29.4 mV). Ethanolic *N. sativa* seed extract loaded ethosomes were successfully prepared by varying SPC concentration and different ethanol: water ratio. Total nine formulations were prepared and evaluated for their entrapment efficiency, vesicle size, Polydispersity Index (PDI) and zeta potential. Formulation E7 prepared with 3% SPC and 20:80 ethanol: water ratio having highest entrapment efficiency. Furthermore, all prepared formulation showed entrapment efficiency in the range between 61.4-78.6%, while vesicle size in the range of 183-193 nm, PDI in the range of 1.1-1.5 and zeta potential between -28.5to -31.7. The optimized transethosomes formulation E7 had entrapment efficiency (78.6±3.1%), vesicle size (188±11 nm), Polydispersity Index (1.3±0.3) and zeta potential (-30.2mV) Table 4& Figure 1-4. Optimized nano-vesicular formulations were further incorporated into carboxyvinyl polymer carbomer (Carbopol 934P) to produce gel formulations. Viscosity, pH and spreading diameter of transethosomal and ethosomal gel formulation was found to be 4400±5.0, 4300±5.0: 6.4±0.3, 6.5±0.3 and54±2.0, 56±2.0 respectively. Drug content of transethosomal and ethosomal gel formulation was found to be 74.1±1.9 and 70.5±2.1 respectively Table 5. *In vitro* drug release through dialysis membrane showed 28.3% and 34.3 %, 14.9% extract released from Te3, GELTe3, EX respectively in 4 hrs, while marketed formulation showed 13.4% drug release. Within 24 hrs total amount of drug release was found to be 97.4%, 99.9%, 56.3% and 44.6% respectively from Te3, GELTe3, Extract and marketed gel. Cumulative percent extract release (CDR) in 24 hr was found to 89.62 %, 98.23%, 47.3% and 42.45 % for Batch Te3, GELTe3, EX& marketed formulation (Angle Gloss, Phyto-lab Pvt. Ltd) respectively. CDR Batch for both T4 and GELT4 was significantly higher than marketed formulation (Angle Gloss, Phyto lab Pvt. Ltd.) ($p< 0.05$) Figure 5& 6. *In vitro* drug release through dialysis membrane showed 26.1%, 32.3 % and 14.9% extract released from E7, GELE7 and EX respectively in 4 hrs, while marketed formulation showed 13.4% drug release. Within 24 hrs total amount of drug release was found to be 95.3%, 98.3%, 56.3% and 44.6% respectively from Te3, GELTe3, EX and marketed gel. Cumulative percent extract release (CDR) in 24 hr was found to 87.54 %, 95.65%, 47.3% and42.45 % for Batch E7, GELE7, EX& marketed formulation (Angle Gloss, Phyto lab Pvt. Ltd) respectively. CDR Batch for both T4 and GELT4 was significantly higher than marketed formulation (Angle Gloss, Phyto lab Pvt. Ltd.) ($p< 0.05$) Figure 7 & 8. Different kinetic equations including zero order, first order, Higuchi's model, Hixson Crowell cube root model and Korsmeyer-Peppas model were tested for all the prepared gels Table 6. Fluorescence microscopy images of prepared nano-vesicular gel formulations showing depositions of fluorescence marker Rhodamine B through (a) GEL Te3 (b) GEL E7 (c) M gel. All formed nano-vesicular gels were white to brown in colour, with homogenous and smooth in texture

Figure 9. Anti-psoriatic activity of ethanolic seed extract of *N. sativa* and extract loaded vesicular gel formulations revealed significant differentiation in epidermis as seen from its degree of orthokeratosis as compared to control. It is similar to the standard tazarotene (0.1%) gel which showed 87.92±2.21% degrees of orthokeratosis. The granular layer is greatly reduced or almost absent in epidermis of psoriatic leisions and this is known as parakeratosis. This parakeratosis is one of the most important characteristic features of psoriasis. Granular layer formation around the epidermis is known as orthokeratosis condition. Conversion of parakeratosis condition to orthokeratosis is the main principle behind the rat tail test. Granular layer formation is indicated by the degree of orthokeratosis. Psoriasis is a disease resulted from the hyper proliferation and abnormal differentiation of keratinocytes. A successful anti psoriatic drug that targets the epidermis is defined as a compound that ideally shows low toxicity and restores skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation, or both. Results concluded that the ethanolic extract of *Nigella sativa* seeds produced well defined granular around the epidermis which is confirmed by its degree of orthokeratosis (68.62±2.43%). The *Nigella sativa* seed extract showed 58.84% activity in the albino model for psoriasis. In relative epidermal thickness, the ethanolic extract of *Nigella sativa* showed significant increase when compared to control group. The degree of orthokeratosis, drug activity and relative epidermal thickness % represented in Table 7. Compared with the total control group animals, in disease control group animals dorsal skin sections showed increased epidermal thickness, elongation of rete ridges and capillary loop dilation. It confirmed the successful development of the disease induced animal model as given in Figure 10 (a). Topical application of standard Tazarotene gel *and N. sativa* extract showed a reduction in thickness of the epidermis, less elongation of rete ridges with capillary loop dilation which observed in Figure 10 (b) and (c) respectively. The animal treated with *N. sativa* extract loaded nano-formulations gel showed comparatively more significant results for reduction in epidermal thickness and less elongation of rete ridges. All of these resulted from the histopathological section mentioned in Figure 11 (a)-(f). However, conventional gel formulation treated animal showed a nominal decrease in epidermal thickness. [Figure 11 (f)] This may be due to enhanced permeation ability of *N. sativa* extract loaded transferosomal gel through the psoriatic skin. Note that granular layer is less developed in most parts of the control specimen (a), Tazarotene induced orthokeratosis are clearly seen over the whole horizontal length of the scale as black layer, marked with an arrow. (b), well developed granular layer is also seen in (c), which is treated with *N. sativa* seeds ethanolic extract. Mass and thickness differences between left and right ear were used to evaluate the induced ear swelling. Digital external micrometer (BAKER DMM25) was used to measure thickness. Ear swelling changes (including ear mass and thickness) took place in right ear lobes of DNCB induced dermatitis animal. Ear swelling changes in right ear lobes were observed in respect to ear mass and thickness. The decrease in ear thickness and reduction in ear mass has been represented as a bar graph in Figure 12(A) & (B). *N. sativa* extract loaded nano-formulation entrapped gels showed remarkable normalization of albino ears. There was no morphological change observed in the untreated left ear lobe. Ear swelling changes (including ear mass and thickness) were observed in right ear lobes of DNCB induced dermatitis animal. The decrease in ear thickness and reduction in ear mass has been represented as a bar graph in Figure. 12. In this study, we observed that the topical application of gels containing *N. Sativa* loaded transethosomes and ethosomes presented significant reduction in ear thickness induced by DNCB, % inhibition of edema induced by carrageenan, decreasing epidermal thickness in IMQ induced psoriasis. These pathological changes were in accordance with the previous literature prepared novel transferosomes of tacrolimus to treat atopic dermatitis induced by DNFB [37]. On comparison with commercial Angle Gloss, topical application of GELTe3 displayed the best therapeutic effect on rat atopic dermatitis. There were no morphological changes observed in the untreated left ear lobe. The prepared transethosomes gel of *N. Sativa* extract loaded was found to be more effective in imiquimod-induced psoriasis; DNCB induced dermatitis and in inflammation as compared to *N. Sativa* extract loaded ethosomal. This may be due to the fact that incorporation of phytoconstituents into vesicular system like transethosomes resulted in much better absorption profile which enables them to cross the biological membrane, resulting in enhanced bioavailability. Hence more amounts of phytoconstituents become present at the site of action at similar or less dose as compared to the conventional plant extract. The therapeutic action becomes enhanced, more

detectable, and prolonged. In comparison with conventional marketed preparation both the extract and phytoconstituents loaded formulation were found to be more effective.

Table 4: Represents entrapment efficiency, vesicle size, polydispersity index (PDI) and zeta potential

Transethosomes					Ethosomes				
F. code	**Entrapment efficiency (%)**	**Vesicle size (nm)**	**Polydispersity Index (PDI)**	**Zeta potential (mV)**	**F.code**	**Entrapment efficiency (%)**	**Vesicle size (nm)**	**Polydispersity Index (PDI)**	**Zeta potential (mV)**
Te1	53.7±2.4	167±9	1.3±0.3	-23.5	E1	61.4±3.1	183±10	1.4±0.4	-29.7
Te2	59.5±2.9	182±10	1.4±0.3	-24.8	E2	63.7±2.9	183±10	1.3±0.3	-28.7
Te3	71.7±2.7	172±11	1.3±0.3	-29.4	E3	66.7±1.8	193±12	1.5±0.3	-30.8
Te4	66.5±2.6	199±12	1.1±0.4	-23.5	E4	69.8±2.8	193±13	1.2±0.3	-31.7
Te5	66.4±2.6	183±11	1.2±0.3	-25.8	E5	71.8±2.8	183±10	1.3±0.3	-28.5
Te6	63.7±2.9	184±12	1.3±0.3	-26.2	E6	77.7±2.9	190±12	1.4±0.3	-29.0
Te7	60.4±2.1	183±12	1.1±0.3	-26.9	E7	78.6±3.1	188±11	1.3±0.3	-30.2
Te8	59.6±2.8	193±12	1.0±0.3	-27.4	E8	72.8±2.9	183±12	1.1±0.3	-29.9
Te9	61.7±2.7	193±10	1.1±0.3	-.28.9	E9	66.5±3.0	190±12	1.3±0.4	-30.1

Table 5: Evaluation of physicochemical properties of *N. sativa* ethanolic seed extract loaded nano-vesicular gel formulations

F.Code	Colour	Homogeneity	Texture	Viscosity (centipoise)	pH	Spreading diameter after 1 min(mm)	Drug content (%)
GELTe3	White to brown	Homogenous	Smooth	4400±5.0	6.4±0.3	54±2.0	74.1±1.9
GELE7	White to brown	Homogenous	Smooth	4300±5.0	6.5±0.3	56±2.0	70.5±2.1

Table 6: Kinetic assessment of release profile of *N. sativa* ethanolic seed loaded nano-vesicular gels

Formulation codes	Zeroorder modelr2	First order modelr2	Higuchi Modelr2	Hixson Crowell model	Korsmeyer&Peppas Modelr(n)
GELTe3	0.831	0.989	0.961	0.954	1.01
GELE7	0.802	0.973	0.945	0.921	1.13
Marketed gel (M gel)	0.891	0.910	0.943	0.891	1.21

Table 7: Effects of ethanolic *N. sativa* seeds extract and extract loaded vesicular gel formulations on the degree of orthokeratosis and relative epidermal thickness as well as the drug activity in the albino rat model

Treatment groups	Degree of orthokeratosis (%)	Drug activity (%)	Relative epidermal thickness (%)
Group 1: Control	16.32± 3.24*	0.0	98.24±9.28
Group 2: Positive Control	56.23±3.63*	0.0	147.59±8.87
Group 3: Nigella sativa seed extract	68.62±2.43*	58.84	135.86±7.56
Group 4	76.26±3.31*	74.82	121.75±5.72
Group 5	79.37±2.89*	68.23	123.75±6.65
Group 6: Standard Tazarotenegel (0.1%)	87.92±2.21*	84.99	104.54±4.20

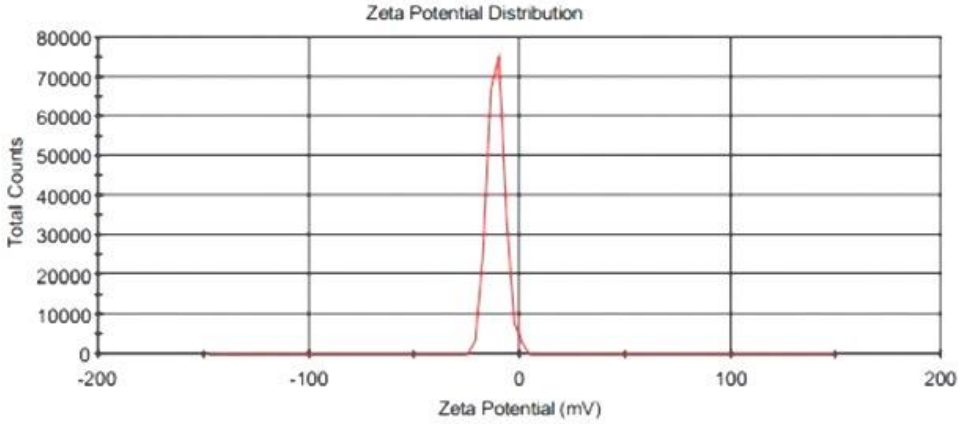


Figure 1: Zeta potential of optimized (Te3) transferosomes formulation

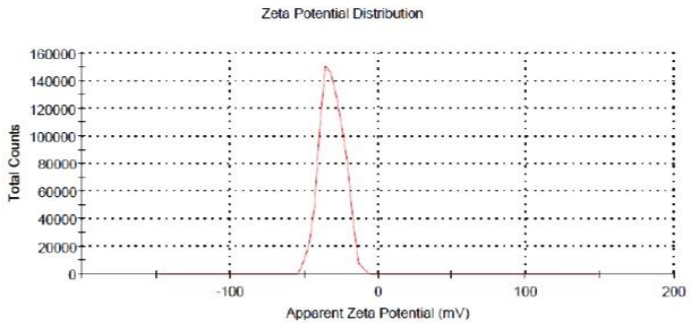


Figure 2: Zeta potential of optimized (E7) ethosomes formulation

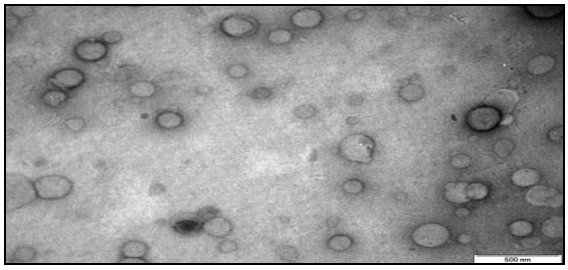


Figure 3: TEM image of optimized (Te3) transethosomes formulation

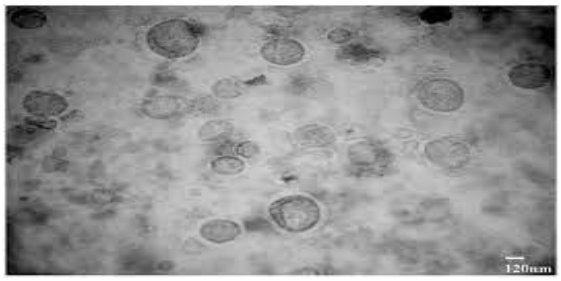


Figure 4: TEM image of optimized (E7) ethosomes formulation

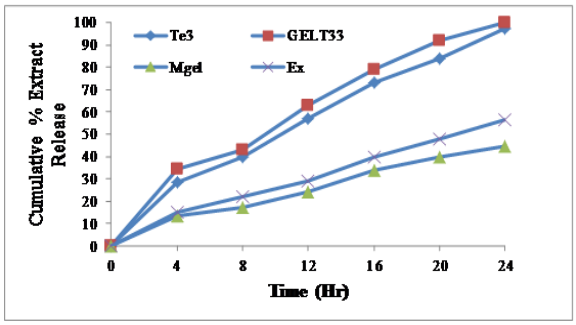


Figure 5: % cumulative drug release after 24 hrs from various transethosomal gel formulations through membrane

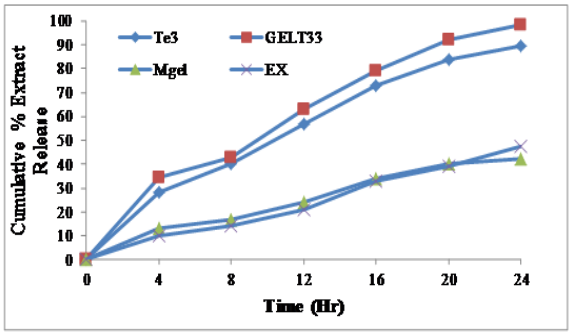


Figure 6: % cumulative extract release after 24 hrs from various transethosomal gel formulations through membrane

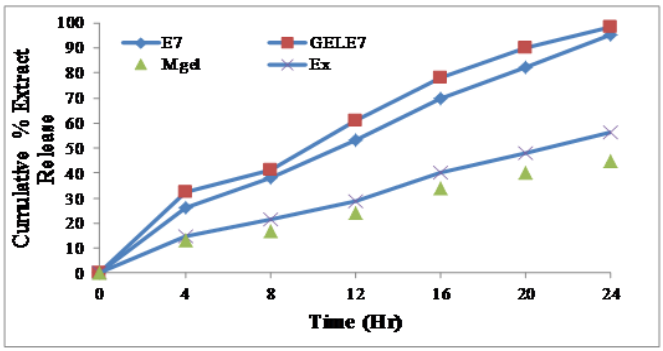


Figure 7: % cumulative drug release after 24 hrs from various ethosomal gel through membrane

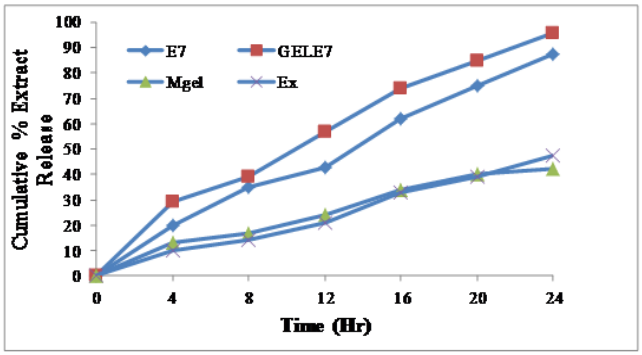


Figure 8: % cumulative extract release after 24 hrs from various ethosomal gel through animal skin

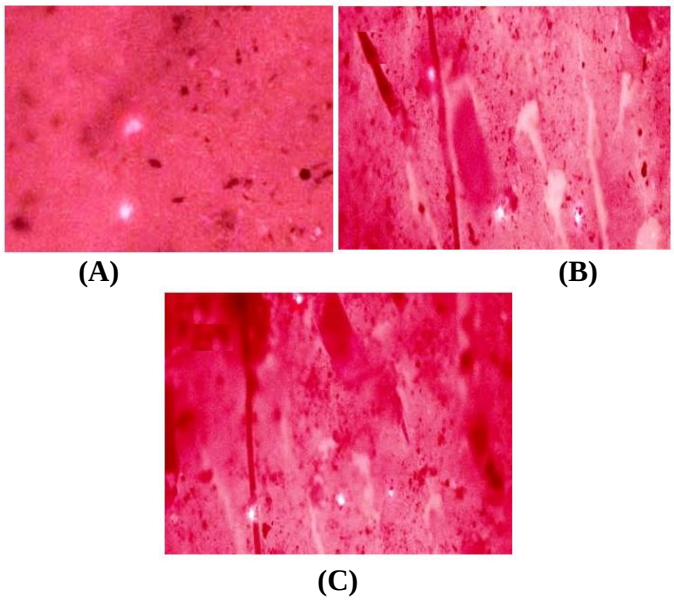


Figure 9: Fluorescence microscopy images showing depositions of fluorescence marker Rhodamine B through (A) GELTe3 (B) GELE7 (C) Mgel

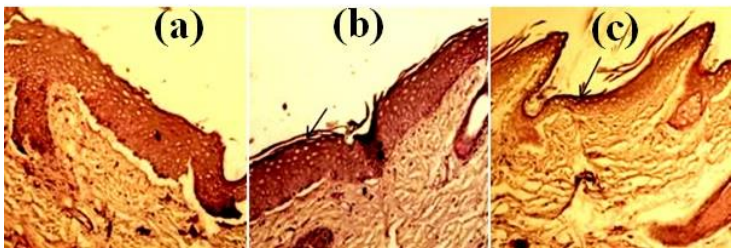


Figure 10: Histopathological sections of mouse tail skin treated topically for 14 days, (original magnification 40×) (a) Control; (b) Tazarotene 0.1%; (c) Ethanolic extract of *N. sativa*seeds

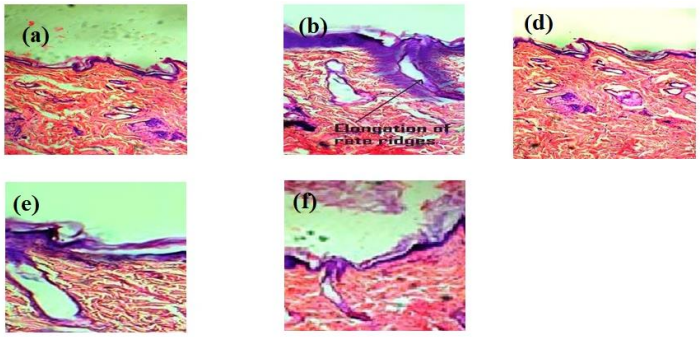
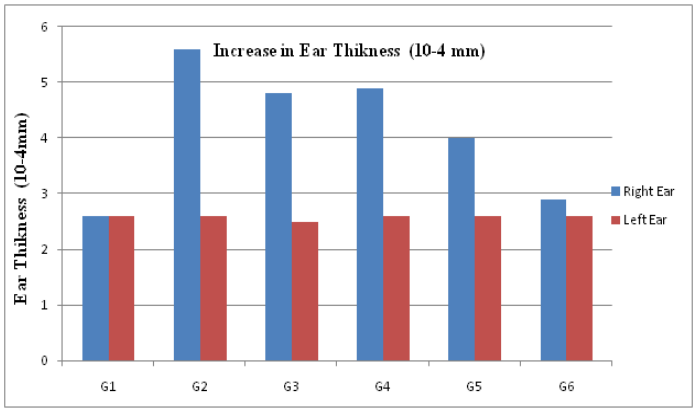
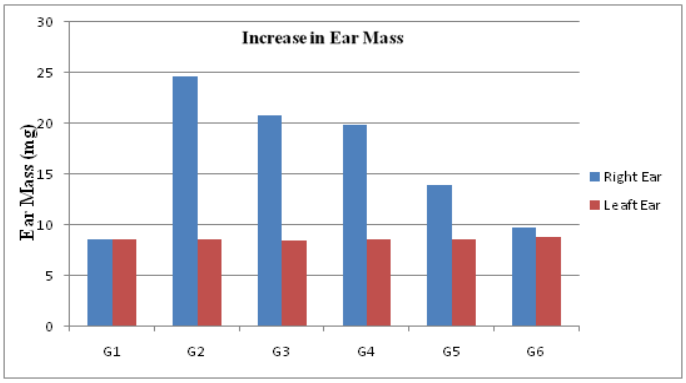


Figure 11: Histopathology images of (a) control animals; (b) Disease control animals; (d) Animal treated with transethosomes loaded gel; (e) Animal treated with ethosomes loaded gel and; (f) Animal treated with commercial formulation (Tazarotene 0.1% gel)



(A)



(B)

Figure 12: Bar graph representation for an increase in ear thickness (A) & ear mass (B)

Conclusion

The results obtained from this study indicates, new phospholipid carrier transethosomes which consists of high concentration of ethanol and edge activator enhances the permeation of *Nigella sativa* ethanolic extract due to its enhanced penetration as compared to ethosomes, and conventional marketed preparation. The prepared transethosomes gel of *N. sativa* extract loaded was found to be more effective in imiquimod-induced psoriasis; DNCB induced dermatitis and in inflammation as compared to *N. Sativa* extract loaded ethosomal. This may be due to the fact that incorporation of phytoconstituents into vesicular system like transethosomes resulted in much better absorption profile which enables them to cross the biological membrane, resulting in enhanced bioavailability. Hence more amounts of phytoconstituents become present at the site of action at similar or less dose as compared to the conventional plant extract. The therapeutic action becomes enhanced, more detectable, and prolonged. In comparison with conventional marketed preparation both the extract and phytoconstituents loaded formulation were found to be more effective. Hence, the elastic formulation transethosomes was found to be more effective as compared to the ethosomes it contains

both ethanol and edge activator which further enhances its transdermal permeation.

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