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Original Article

Development and Assessment of Phytophospholipid Nanovesicular Systems for Treatment of Diabetic Neuropathy

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ABSTRACT

Background: Diabetic neuropathy (DN) is the most prevalent and problematic consequence of diabetes mellitus, with the highest rates of morbidity and death, as well as a significant financial burden on diabetes treatment. Aside from commercially available formulations, substantial research on herbal-based natural therapies to treat hyperglycemia and related consequences is underway. **Objectives:** The major aim of the study was to use an ethosomal gel to improve transdermal penetration of *Curcuma longa* and *Boswellia serrata* and to overcome the challenges of low transdermal drug delivery permeability. **Materials and Methods:** *Curcuma longa* and *Boswellia serrata* loaded ethosomes were made using a cold process with various amounts of ethanol and soya lecithin, and were improved based on entrapment efficiency, vesicular size, and *ex-vivo* investigations. **Results:** The optimized ethosomal formulation was then put into a gel and evaluated. *Ex-vivo* tests were conducted with *Curcuma longa* and *Boswellia serrata* ethosomal gels, which were compared to plain gel. The ethosomal system produced vesicles with sizes ranging from 1 to 100 nm, a spherical, smooth surface, and a maximum entrapment efficiency of Ne9. A modified ethosomal system (Ne9) was introduced into the gel and studied further. **Conclusion:** According to the findings, the ethosomal gel has a lot of potential for transdermal *Curcuma longa* and *Boswellia serrata* administration.

Keywords: *Curcuma longa*, *Boswellia serrata*, Ethosomes, Ethosomal gel, *Ex-vivo* studies, Drying studies.

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INTRODUCTION

Diabetic neuropathy is the most common microvascular complication of diabetes, and it is associated with severe morbidity and mortality. Diabetic neuropathy (PDN) is the most frequent cause of neuropathic pain, affecting 20% to 25% of diabetics.[1] Creams containing capsaicin, citrullus colocynthis, and other phenolic chemicals are among the topical treatments available. However, because the active ingredient's bioavailability is low and epidermal denervation has been recorded in capsaicin-treated individuals, it should be taken with caution. Other herbal medicines commonly include volatile oils, which evaporate quickly and do not generate a long-lasting impact, necessitating the development of a formulation that can produce both a long-lasting effect and the intended action.[2] Most herbal drugs, on the other hand, are insoluble, resulting in lower bioavailability and higher systemic clearance, necessitating repeated administration or a higher dose, making the substance a poor therapeutic option. Herbal extracts, which are normally non-habit forming, can be included into new formulation processes for additional advantages including bulk dose and bypassing the principal barrier of absorption. For herbal drugs, developing nano dosage forms (Polymeric Nanoparticles, Nanospheres and Nanocapsules, Liposomes, Proliposomes, Solid Lipid Nanoparticles (SLNs), Nanoemulsion, etc.) has a number of advantages, including improved solubility and bioavailability, toxicity protection, pharmacological activity enhancement, stability enhancement, improving tissue macrophage distribution, sustained delivery, and protection from physical and chemical degradation.[3] Recognizing this issue, the authors devised herbal ethosomes that had a long-lasting impact.

In the traditional Indian medicinal system, plant mixtures and blended extracts of plants are valued above individual ones. Ayurvedic herbals are known to come in a range of dosage forms, with Poly herbal formulations accounting for the bulk of them. The presence of numerous phytoconstituents in herbal remedies has a therapeutic effect, and the advantages are magnified when appropriate herbals are combined in a Polyherbal formulation. It is usually recognised that they have a wide therapeutic range. They offer a higher risk-benefit ratio since the majority of them are effective at low doses and safe at high levels.[4-5]

The ethosomes used in herbal remedies can readily penetrate the deepest layers of the epidermis

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as well as the systemic circulation. Other transdermal or cutaneous medication formulations have benefits over herbal ethosomes.[6] They have a high permeability capacity, and big molecules such as proteins and peptides may be easily employed in their formulation, making ethosome a superior alternative for drug administration that can easily permeate the epidermal layers.[7] They entrap a wide range of pharmacological compounds, including hydrophilic, lipophilic, and amphiphilic molecules. It is prepared using permeability enhancers. When iontophoresis, phonophoresis, and ethosomes are compared, ethosomes are the simplest route of drug administration.[8] Because ethosomes contain a significant amount of ethanol, their lipid membrane is less densely packed than other vesicles, yet they are extremely stable. It was discovered that when an herbal ethosome was created, the ethosome preparation increased medication transport through the stratum corneum into the deeper layers of the skin.[9]

The use of ethosomes, a noninvasive drug carrier that reaches the deep epidermal layers or the blood circulation, is a simple and practical way to administer drugs. Ethosomes are soft, flexible vesicles that aid in the distribution of medications. These ethosomes have a high rate of penetration into the skin due to the use of ethanol as a penetration enhancer.[10] Ethosomes help to improve drug efficiency, patient compliance, and comfort while also cutting expenses. Enhanced solubility and bioavailability, toxicity protection, pharmacological activity enhancement, stability enhancement, improved tissue macrophage distribution, sustained delivery, protection from physical and chemical degradation, and other advantages of ethosomal gels for herbal drugs are just a few of the advantages.[11]

Our goal was to create a polyherbal ethosomal gel that would increase lipid solubility and reduce molecular size of herbal extracts, allowing medication molecules to pass through the biological membrane and absorb systematically after topical application, hence increasing bioavailability.

MATERIALS AND METHODS

Materials

Curcuma longa and *Boswellia serrata* were obtained from a local legitimate herbal distributor in Kanpur, Uttar Pradesh, identified at Pranveer Singh Institute of Technology, Kanpur, and phytochemically analyzed using the spectrophotometric technique. Changshuyangyuan chemicals china/ central pharmaceuticals house, New Delhi, provided ethanol, methanol, and triethanolamine. SD fine chemicals limited, Mumbai, provided methyl paraben, pet ether, vanillin, and toluene. SD fine chemicals limited, Mumbai, provided carbopol 934, soy lecithin, and propylene glycol.

Methods

Extraction of Herbs

It's a method of extracting a desired ingredient from a mixture or raw material. *Curcuma longa* and *Boswellia serrata* were used to extract chemical ingredients for our investigation.[12]

Curcuma longa extraction

Curcuma longa dried roots acquired from a local Kanpur shop. After passing it through sieve #60, the roots were crushed and the proper particle size was collected. The soxhlet apparatus was used to conduct solvent extraction with pet-ether as the solvent. Rotavapour was used to remove the solvent after extraction. The resulting oil should be packaged in aluminium foil in a glass jar and stored in the refrigerator at 9-10°C until needed.[13]

Boswellia serrata extraction

Boswellia serrata oleo gum resin obtained from India Mart's official website. The plant's oleogum resin was combined with hot distilled water on a magnetic heater for 1.5 hr, after which the extract was filtered. Rotavapour was used to remove the solvent. The porous powder that resulted was kept in the refrigerator at 4-8°C until needed.[14]

Thin Layer Chromatography (TLC)

It's a chromatographic method for separating non-volatile chemicals in a mixture. TLC plates were made using silica gel GF 250 for the validation of the synthetic process and product identification. Distilled water was used to make a silica gel slurry, which was then put on TLC glass plates and baked for 30 min at 30°C. UV and iodine chambers were used for visualization.[12]

Chemical Test of *Curcuma longa* Extract

Test for tannins: Five millilitres of extract were mixed with five millilitres of distilled water, and a few drops of ferric chloride solution were added.

Test for saponins: 2 mL extract was heated after being treated with 2 mL distilled water.

Test for alkaloids: 2 ml extract and 3 ml 1 percent HCl were cooked on a steam bath, and 1 ml of the mixture was divided into two test tubes, with a few drops of Dragendorff's reagent in one tube and Mayer's reagent in the other.

Test for flavonoids: One millilitre of extract was treated with one millilitre of a 10% lead acetate solution. The presence of alkaloid was shown by a yellow precipitate.

Test for terpenoids: 2 mL extract was mixed in 2 mL CHCl3 and evaporated to dryness before adding 2 mL conc. H_2SO_4 and heating for 2 min.[12]

Powder Properties of *Curcuma longa* and *Boswellia serrata* extract[9,15]

Bulk Density

In a 100 ml measuring cylinder, 5 g of herbal extract was inserted. Following that, the bulk volume was measured and the bulk density was determined using the following formula:

$$\text{Bulk density} = \frac{\text{Mass}}{\text{Volume}}$$

Tapped density

It is achieved by mechanically tapping a measuring cylinder containing a large volume of product for three minutes. The tap volume was then recorded, and the tapped density was computed using the following formula:

$$\text{Tapped desnity} = \frac{\text{Mass}}{\text{Volume}}$$

Compressibility

It is capacity of powder to be flattened or reduced in size by applying mechanical pressure it is calculated by given formula:

$$\%\ \text{Compressibility} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's Ratio

It is a number which is correlated to the flowability of a powder and a granular material. It is calculated by given formula:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of Repose

A funnel was mounted on a tripod platform, with a graph paper put beneath it, and powdered substance was poured down the funnel to form a cone. The funnel tip should be near to the developing cone and gently lifted; pouring should cease when the growing cone touches the funnel tip. The angle of repose was computed using the following formula:

$$\tan\theta = \frac{h}{r}$$

Partition Coefficient of Extracts

In a separating funnel, 10 mL N octanol and 10 mL phosphate buffer pH 6.8 were combined for 60 min. *Curcuma longa* and *Boswellia serrata* extracts (1 mL each) were added to the mixture and agitated for 30 min. The partition coefficient was measured using a UV visible spectrophotometer after the organic and aqueous layers were separated.[16]

UV Spectrophotometer Study

Curcuma longa and *Boswellia serrata* stock solutions were produced in phosphate buffer pH 6.8 at a concentration of 1000 µg/ml each. 2-10 µg/ml concentrations were made from the stock solution, and UV spectrophotometry was used to scan the wavelength range of 200-400 nm. Then, for *Curcuma longa* and *Boswellia serrata*, different concentrations were exposed to UV for determination of absorbance at scanned max.[17]

Method of Preparation of Ethosomes

The cold approach was used to make polyherbal ethosomes. Phospholipid and lipophillic herbal extract were mixed in ethanol at room temperature using a mixer and vigorous shaking. During the stirring process, 5 mL of propylene glycol was added. The produced mixture was heated in a water bath to 30°C± 1°C while being constantly mixed at 700 rpm with a mechanical stirrer. In another jar, water was added to hydrophilic extract and heated to 30°C± 1°C. In a covered beaker, aqueous phase was introduced one drop at a time into non-aqueous phase while stirring for 5 min (Table 1). The formulation was allowed to cool at ambient temperature for 30 min before being sonicated at 40°C for five cycles of three minutes each with a one-minute pause between cycles to achieve the appropriate ethosome size.[15,18] The complete formulation was then stored in the refrigerator.

Percentage Drug Entrapment Study

2 ml of ethosomal suspension was placed in a 10 ml volumetric flask and combined with a suitable amount of phosphate buffer pH 6.8 to determine extract entrapment. The mixture was centrifuged for 25 min at 1500 RPM, and the supernatant liquid layer was collected. The UV-1700 Shimadzu was used to evaluate the absorption of 1 mL supernatant liquid combined with 9 mL methanol. For *Curcuma longa* and *Boswellia serrata*, the UV range was retained at 423 nm and 260 nm, respectively.[19] The drug entrapment efficiency calculated by given formula:

$$\text{Dee}\,(\%) = \frac{(\text{Wt} - \text{Wf})}{\text{Wt}} * 100$$

Table 1: Formulation design of ethosomes.

Formulation code	Drug ratio (C*: B**)	Soya lecithin %w/v	Ethanol (ml)
Ne1	2:1	0.9	20
Ne2	2:1	0.9	30
Ne3	2:1	0.9	40
Ne4	2:1	1.2	20
Ne5	2:1	1.2	30
Ne6	2:1	1.2	40
Ne7	2:1	1.5	20
Ne8	2:1	1.5	30
Ne9	2:1	1.5	40

*= *Curcuma longa*; **= *Boswellia serrata*

DEE = Drug entrapment efficiency, Wt = Total ammount of ethosomes, Wf = Free amount of ethosomes in Supernatant.

Characterization of Ethosomes

Scanning electron microscopy (SEM)

SEM focuses on the surface and composition of the sample and provides information on the morphology of the sample. A three-dimensional picture is also produced using SEM. Jeol's scanning electron microscope model no. JSM-6490LV from Babasahab Bhimrao Ambedkar University, Lucknow, was used to examine the surface morphology of produced ethosomes.[20]

Fourier-transform infrared spectroscopy (FTIR)

Infrared spectroscopy examinations reveal the presence of any functional groups in an unknown chemical. We used a Perkin Elmer FTIR spectrometer to identify functional group for this work. All formulations were examined in the 4000-400 cm^{-1} wavelength range.[21]

Zeta potential

Because it is a significant indication for the particle surface charge, it is utilized to determine and control the stability of suspension particles. The ethosomes all had negative zeta potential values ranging from -11.4 mV to -29.6 mV. The optimized formulation NE8 had the highest negative zeta potential, which was considered favourable for formulation stability and drug transdermal permeation enhancement due to electrostatic repulsion between skin surfaces with the same charge, implying that the formulated ethosomes do not aggregate rapidly. The charge of vesicles is a key factor that affects both stability and the interaction of skin vesicles.[22]

In-vitro Release

The amount of herbal extracts released at a specific moment was determined by examining ethosomal suspensions. The ethosomes were studied *in vitro* using a Franz diffusion cell with a diffusional area of 0.075 cm^2. First, a magnetic bead was put in the receptor compartment, followed by the placement of an egg membrane between the donor and receptor compartments of the Franz diffusion cell, which was then sealed with springs. Phosphate buffer pH 6.8 was given to ethosomes in the donor and receptor compartments. At 250 RPM, a magnetic bead was used to agitate the phosphate buffer constantly. One ml sample was withdrawn at every 1 hr. interval and 1 ml fresh buffer was added at every withdrawal. Absorbance of all samples were measured by UV-1700 Shimadzu 423 nm and 260 nm for *Curcuma longa* and *Boswellia serrata* respectively.[23]

Table 2: Carbopol gel of optimized ethosomal suspensions.

Formulation code	Ethosomal suspension (ml)	Carbopol (%w/v)	Glycerol (%w/v)	Triethanolamine (%w/v)	Benzalkonium chloride (%w/v)	Water
Ne8	10	1	6	0.2	0.01	q.s.
Ne9	10	1	6	0.2	0.01	q.s.
Plain gel	-	1	6	0.2	0.01	q.s.

Formulation of Ethosomal Gel

Zeta potential, entrapment efficiency, and release experiments were used to improve ethosomal suspensions. Carbopol 934 was used to convert optimized ethosomal suspensions (Ne8, Ne9) into gel. Carbopol 934 was swelled overnight after being distributed in distilled water at 300 rpm for 2 hr. After one day, dropwise additions of improved ethosomal suspension to carbopol were made with continuous stirring to generate a homogeneous mixture. Triethanolamine was added until a clear gel was achieved (Table 2). As a preservative, 0.01 percent benzalkonium chloride was used to defend against bacteria and fungus.[20]

Evaluation of Gel

Viscosity

Viscosity of ethosomal gels was carried by Brookfield Viscometer (RVDV-II+PRO) by selecting spindle ≠ 5. Prepared ethosomal gels were taken into 50 ml beaker. Spindle was dipped into beaker and viscosity was measured at 20, 50 and 100 rpm.[20]

Determination of gel pH

pH of ethosomal gel formulations were carried out by using digital pH meter. 1 g of ethosomal gel was dissolved in 100 ml of distilled water and kept for 1 hr. The pH of prepared gel formulations was measured.[20]

Spreadability

A spreadability study was conducted to determine the quantity of ethosomal gel spread across a glass slide at a given period. The gel's therapeutic impact is also influenced by the spreadability of the produced formulation. It is measured in seconds needed by two slides to slip from a gel that is put between them in a continuous direction with a specific amount of weight.[24] It's computed using the following formula:

$$S = \frac{M * L}{T}$$

Where M is weight which used slide the upper slide, L is length of glass slide, and T is time taken to the separate the slide.

Extrudability study

The amount of gel extruded from a collapsible tube in percentages was calculated using the weight in grams necessary to extrude at least a 0.5 cm ribbon of gel in 10 sec in the current investigation.[30] The extrudability was then calculated by using the following formula:

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (gm)}}{\text{Area (cm}^2\text{)}}$$

Homogeneity

After the gels were set in the container, they were visually inspected for uniformity. They were examined for the existence of aggregates and their appearance.[31]

Ex vivo gel release

Following anesthetization, rats were sacrificed, the abdominal fur was removed with a razor and the skin was carefully excised and washed with normal saline (1273/PO/Re/S/09/CPCSEA). A circular piece of skin in contact with the receiver medium and the epidermis side in contact with the donor chamber was securely sandwiched between the receptor and donor compartments with the dermal side (contact area= 0.75 cm^2). 1 gm ethosomal gel has been placed in intimate contact with the skin excised. The donor compartment was charged with an adequate sample amount to keep the drug quantity constant and the receptor compartment was filled with 20 ml of pH 6.8 phosphate buffer saline and stirred at room temperature with a magnetic stirrer at 300 rpm. The samples were withdrawn at different intervals of time, filtered, adequately diluted and then analyzed at 423 nm and 260 nm for *Curcuma longa* and *Boswellia serrata* respectively using a UV spectrophotometer and replaced with the same fresh buffer volume. Kinetic study of release data was performed concerning zero-order, First order, Higuchi model and Korsemeyer–peppas. The best fit model was confirmed by the value of the determination coefficient near one.[25] The permeation flux for ethosomal gel and plain gel was determined. Permeation flux is the slope of percentage drug release v/s time. It is expressed as µg/cm^2/hr.

Drying rate of ethosomal gel

Drying experiments were carried out in a vacuum tray dryer where the temperature and vacuum could be maintained independently. An accurate amount of ethosomal gel was taken in a petridish and kept in an oven at the temperature of 50-60°C. Weight of the sample was measured at every 10 min interval and recorded. The data of moisture removed with respect to time was used to evaluate the drying characteristics.[15]

RESULTS

Characterization of Extracts

TLC was performed using 9:1 ratio of Toluene: Ethyl Acetate; Spray component was Vaniline, 0.25gm in 40 ml H_2SO_4 + 10 ml Ethanol. R_f for *Curcuma longa* and *Boswellia serrata* was 0.54 and 0.75 respectively which was found to be similar with reported value. Formation of orange red precipitate, buff colored precipitate and a yellow precipitate indicated presence of alkaloid. While formation of green precipitate and grayish color indicated presence of tannins and terpenoids respectively. Formation of stable foam indicated presence of saponins. Partition coefficient was found to be 3.24 and 1.68 for *Curcuma longa* and *Boswellia serrata* respectively.[16]

Micromeritic study of Herbal extracts

In a free-flowing powder bulk and tapped densities were closer in value and interactions are less significant. The differences are reflected in the compressibility index and hausner's ratio (Table 3). Angle of repose was found to be 25° and 27° for *Curcuma longa* and *Boswellia serrata* respectively. Micromeritic values of herbal extracts revealed good flow properties, angle of repose and all properties properly determined.[17]

Table 3: Organoleptic properties of powder.

Sl. No.	Properties	*Curcuma longa*	*Boswellia serrata*
1	Bulk density (g/ml)	0.9	0.28
2	Tapped density (g/ml)	0.6	0.23
3	Hausner's ratio	0.67	1.12
4	Compressibility	11.58	13.04
5	Angle of repose	25°	27°

Standard curve of *Curcuma longa* and *Boswellia serrata*

Linearity was studied by preparing standard solutions at different concentration levels and by plotting the absorbance against concentrations of the analyte. The regression coefficient value was found to be 0.999 and 0.9985 for *Curcuma longa* and *Boswellia serrata* (Figure 1).

Evaluation of Herbal Ethosomes

The maximum entrapment efficiency obtained was 88.73% and 83.87% for *Curcuma longa* and *Boswellia serrata* respectively for formulation Ne9 containing 1.5 gm of soy lecithin and 40 ml ethanol. Ne2 ethosomal suspension demonstrated minimal entrapment efficiency which was seen as 38.89% (for *Curcuma longa*) and 43.78% (for *Boswellia serrata*) (Figure 2). Ethosomes appear spherical shaped, and no irregularities of aggregation were observed as shown in the Figure 3 (Ne9). Ethosomes gave an average size of 150 to 200 nm in an ideal condition.

In the FTIR spectrum of *Curcuma longa*, the functional groups such as hydroxyl group, carbonyl group and the ethylene group showed peaks

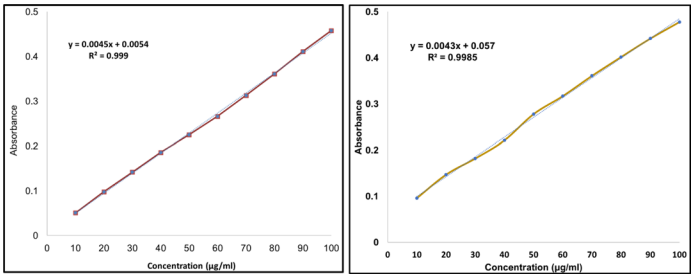

Figure 1: Standard curve of *Curcuma longa* and *Boswellia serrata* at 423 nm and 260 nm respectively.

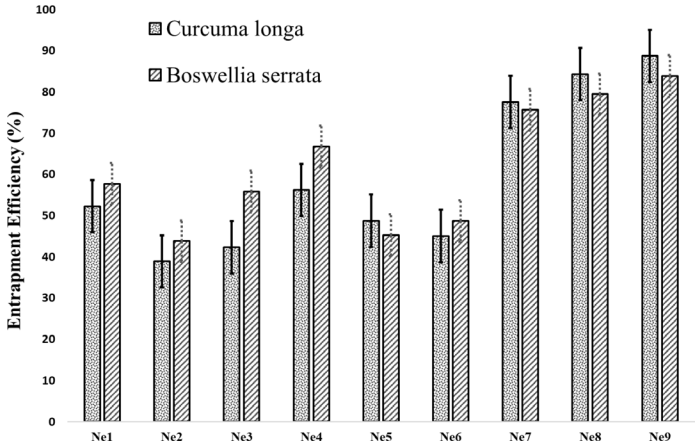

Figure 2: Entrapment efficiency of ethosomal formulations.

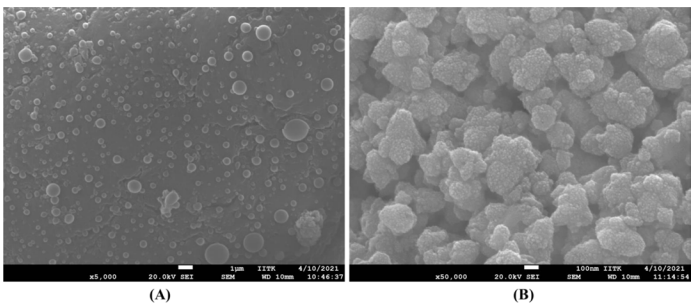

Figure 3: SEM images of Ne8 ethosome at (A): 5000 x, (B): 50,000 x.

Table 4: FTIR spectral vibrations of *Curcuma longa* extract.

Sl. No.	Functional groups along with mode of vibrations	
	Curcuma longa	***Boswellia serrata***
1	3401.4, Stretching vibrations of O-H	3784, -NH_2
2	2923.6, Stretching vibrations of C-H	3605, -OH
3	1679.2, Stretching vibrations of C=O	2367, CH_2-CH_2 (symmetric)
4		1574, -C-C (Aromatic)
5	1616.1, Stretching vibrations of C=C (Alkenes)	1427, COO^- symmetric stretching of carboxylates
6		
7	1442.9, Stretching vibrations of C=C (Aromatic compounds)	1269, C-CO-C stretching of aryl ketone
8		
9	1106.8, Stretching vibrations of C-O 1033.3, Stretching vibrations of C-O 881.03, -HC=CH-(cis) out of plane 723.51, -(CH_2)n ; -HC=CH- bending	1054 and 902, ring structures of cyclohexane

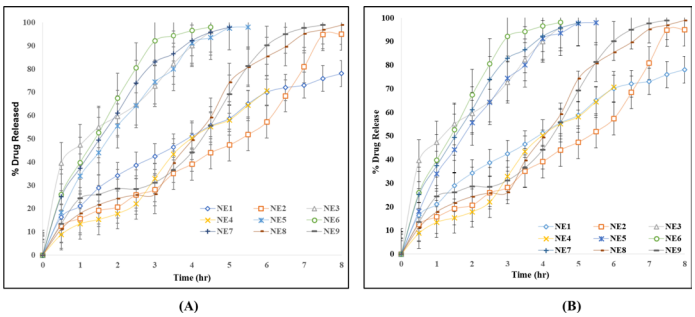

Figure 4: *In vitro* release of (A) *Curcuma longa* and (B) *Boswellia serrata* extracts from ethosomal formulation.

at 3401.4 cm^{-1}, 1616 cm^{-1} and 1442.9 cm^{-1} respectively. In the spectrum of *Curcuma longa*, the peaks at 723.51 cm^{-1}, 812.77 cm^{-1} and 881.03 cm^{-1} indicated the bending vibrations of -CH bond of alkene group. For the *Curcuma longa* incorporated ethosomes, the peaks corresponding to these functional groups were observed at 3301 cm^{-1}, 1640 cm^{-1} and 1418 cm^{-1} respectively (Table 4), which indicates that the major peaks of *Curcuma longa* were retained in the case of *Curcuma longa* incorporated ethosomes also. Both *Curcuma longa* and *Curcuma longa* incorporated ethosomes showed a peak around 1227 cm^{-1}, which corresponds to the C-O stretching frequency of ether group in *Curcuma longa*.[12] The absorption around 1500-1400 cm^{-1} indicated the –C-O elongation frequency of – OH groups in *Curcuma longa* and *Curcuma longa* incorporated ethosomes. The spectrum of pure *Boswellia serrata* gum resin showed characteristic peaks at 3605 cm^{-1} (OH stretching), 2367 cm^{-1} (C-H stretching), 1574 cm^{-1} (C-C Aromatic), 1427 cm^{-1} (COO^- symmetric stretching of carboxylates), 1240 cm^{-1} (C-CO-C stretching of aryl ketone), 1025 cm^{-1} and 988 cm^{-1} (ring structures of cyclohexane). It was observed that there were no significant changes in the position of the characteristic peaks of *Boswellia serrata* was found during manufacturing process of ethosomes.[18] All of the ethosomes exhibited negative zeta potential values in the range of -11.4 mV to -29.6 mV.

In-vitro drug release and kinetics study of ethosomal formulations

In-vitro drug release study of herbal extracts loaded ethosomal suspensions were conducted for a period of eight hour using franz diffusion cell and phosphate buffer saline pH 6.8. The release profiles of entire ethosomal suspension were shown in (Figure 4). Cumulative

Table 5: Kinetic models of ethosomal gels.

Formulations	Zero order (r^2)		First Order (r^2)		Hixson Crowell (r^2)		Higuchi (r^2)		Korsemeyer- peppas			
									T		B	
	T	B	T	B	T	B	T	B	(r^2)	n	(r^2)	n
Ne1	0.9637	0.9539	0.0641	0.4911	0.7996	0.4301	0.9884	0.9809	0.9936	0.6	0.994	0.79
Ne2	0.9538	0.9702	0.0331	0.3445	0.7671	0.4945	0.8423	0.9716	0.9206	0.7	0.9957	0.73
Ne3	0.9009	0.9908	0.1743	0.4019	0.9732	0.4278	0.9826	0.9363	0.9564	0.4	0.9962	0.90
Ne4	0.9691	0.9522	0.1148	0.1635	0.5906	0.4294	0.8832	0.9648	0.9415	0.9	0.9789	0.80
Ne5	0.9471	0.9324	0.092	0.5493	0.884	0.477	0.9812	0.9146	0.989	0.7	0.9714	1.0
Ne6	0.9182	0.8779	0.1469	0.5726	0.8698	0.3994	0.9758	0.9301	0.9819	0.6	0.9394	0.92
Ne7	0.927	0.9671	0.1224	0.4449	0.5419	0.4299	0.9859	0.9508	0.9927	0.6	0..9908	0.88
Ne8	0.9809	0.8576	0.0139	0.7404	0.8011	0.4883	0.8851	0.9365	0.9308	0.7	0.9513	0.77
Ne9	0.9537	0.8678	0.024	0.7128	0.7955	0.4909	0.856	0.928	0.893	0.8	0.9543	0.85

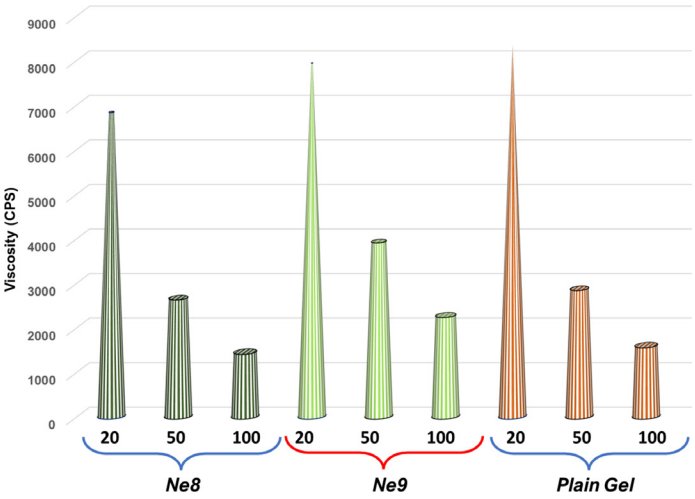

Figure 5: Viscosity of GNe8 ethosomal gel at 20, 50 and 100 rpm.

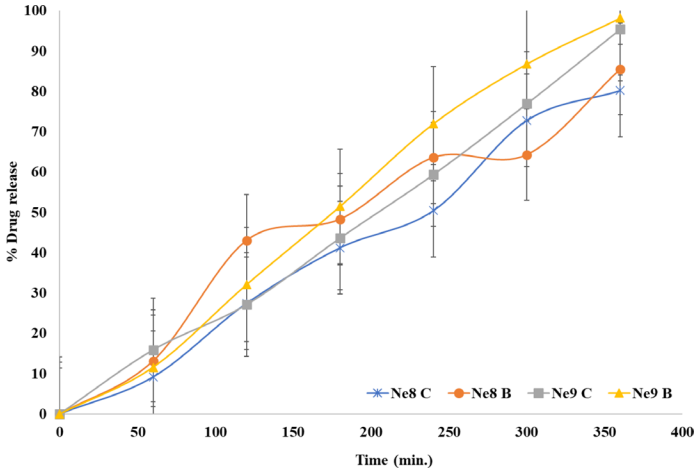

Figure 6: *Ex vivo* release of herbal extracts from ethosomal gels.

amount of drug release was calculated for each formulation. Results revealed that Ne9 had the highest cumulative amount of drug release (98.98% and 99.82%) up to 8 hr for *Curcuma longa* and *Boswellia serrata* respectively. On the other hand, Ne2 showed lowest drug release (70.73% and 86.12%) for *Curcuma longa* and *Boswellia serrata* respectively. The drug release profile was fitted in various kinetics models. Higher the correlation coefficient was judged to be more appropriate model for dissolution data. Kinetics analysis of in vitro release data and the mechanism of drug release was estimated after fitting the obtained data to different kinetics models; zero order, first order, higuchi, hixson-crowell and korsemeyer-peppas. Table 5 also summarizes the values of n of the studied formulations. As the table shows n value was found to be 0.5<n<1 which indicates non fickian diffusion mechanism. However, in formulation Ne9 n ≈ 1 indicates a Case II transport (zero- order).[19]

Gel Characteristics of Formulations

Viscosity is very important in terms of stability and application. According to the findings, the formulations' viscosity rose when the shear rate was raised, indicating pseudoplastic behavior. With increased shear stress, the polymer chains disorganized to fit in the course of the stream, and this orientation reduced the material's internal resistance, allowing for a larger shear rate with each successive shearing stress (Figure 5). As a result, the rheological properties matched and met the requirements of the topical delivery formulations' ideal necessity.[20,18]

The pH values of ethosomal gels were found around 6.4 which is compatible with a normal pH range of skin. Because the gel's therapeutic efficiency is determined by its spread, the spreadability of the optimized gel was determined to be high by having a short time spread. The gel's spreading ability aids in uniform application to the skin (12.50 and 13.63 gm.cm/sec for Ne8 and Ne9 ethosomal gels respectively), and the texture was also deemed smooth. Extrudability of gels was assessed because high consistency gels may not extrude from tubes, whereas low viscosity gels may flow fast, necessitating the employment of a sufficient consistency to extrude gels from tubes.[21] The gel formulation's extrudability was determined to be outstanding. Between the thumb and index finger, a little amount of ethosomal gel was squeezed. When a tiny amount of the ethosomal gel was rubbed on the back of the hand, the homogeneity could also be noticed. In the same way, the grittiness of the produced ethosomal gel was examined.

Ex-vivo drug permeation studies were conducted for ethosomal gels (Ne8, Ne9) for 8 hr using franz diffusion cell on skin pH to evaluate release parameters. The amount of extracts that permeated through the skin after 8 hr from Ne8 and Ne9 was 80.17%, 85.49% and 95.41% and 98.15% for *Curcuma longa* and *Boswellia serrata* respectively (Figure 6). For Ne9, Ne8, and plain gels, flux was determined to be 24.12 gm/cm^2/hr, 19.76 gm/cm^2/hr, and 6.94 gm/cm^2/hr, respectively (Table 6). The capacity of ethosomal gel to squeeze themselves through the comparatively smaller pores of skin explained the greater skin penetration of extracts from ethosomal gel.[22-24]

Table 6: Physicochemical characterization of prepared ethosomal gels.

S. No	Formulation Code	pH	Extrudability*	Homogeneity	Spreadability[a] (gm.cm/sec)	*In-vitro* skin permeation flux[a] (g/cm²/hr)	Shelf Life ($t_{10\%}$)
1.	Ne9	6.4±0.654	+++	Homogeneous	13.68±2.102	24.12±1.091	1 year 5 months
2.	Ne8	6.8±0.911	+++	Homogeneous	12.50±1.654	19.76±1.204	1 year 8 days
3.	Plain gel	5.9±0.233	++	Homogeneous	8.09±0.091	6.94±1.981	-----

*+++ = Excellent; ++ = Good; [a]Values are represented as mean± SD (n=3)

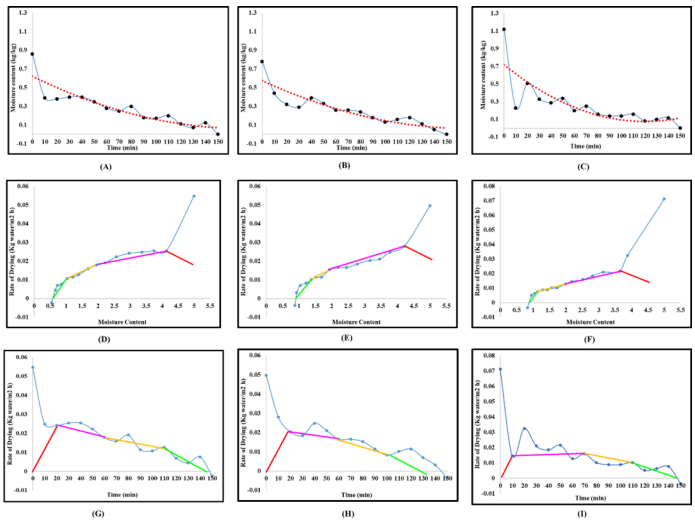

Figure 7: Moisture content vs time data of GNe8 (A: 2% w/v, B: 2.5% w/v, C: 3% w/v); Characteristic drying rate curve (Time independent or Krischer curve) of GNe8 (D: 2% w/v, E: 2.5% w/v, F: 3% w/v); Drying rate vs time plot of GNe8 (G: 2% w/v, H: 2.5% w/v, I: 3% w/v).

Drying Rate of Ethosomal Gels

In a regular drying rate period, gel contains a lot moisture that liquid surface exists will dry in a manner corresponding to an open-faced frame of moisture. Diffusion of moisture from inside the droplet keeps saturated surface conditions and so long as these lasts, evaporation takes area at constant rate. When the gel is dried under constant drying conditions, the water content usually drops. The graph is linear at first, then curves and finally flattens. The drying period of the constant rate lasts until free moisture emerges from the surface, the rate of removal of moisture then decreases. The moisture content at which the drying rate stops being constant is known as critical moisture content (CMC). During a period of constant drying, water vapor from the inside migrates to the surface in various ways and is evaporated. When the moisture level is lowered, the rate of migration to the surface is lowered. CMC of gel formulation (Ne9) was 1.93, 1.93 and 1.98 for 2%, 2.5% and 3% w/v concentration of carbopol 934 gel. The maximum constant rate period is found for 2% and 3% w/v gel, which shows all moisture evaporation. The constant rate period ends when the rate of moisture migration from the inside of the surface becomes less than the rate of evaporation from the surface. The period after the critical point is called the 'fall rate period'. Outside this point, the surface temperature rises, and the drying rate drops rapidly. The falling rate period takes much longer than the constant rate period, although the removal of moisture may be much less. The drying speed is close to zero at some equilibrium moisture content (EMC). The concept of EMC is important in the study of formulation drying and storage. This determines the maximum amount of moisture that the formulation can absorb during storage (Figure 7). EMC for 2%, 2.5% and 3% w/v gels were 0.61, 0.91 and 0.91 respectively.

DISCUSSION

Chemical tests of *Curcuma longa* and *Boswellia serrata* were performed to determine the presence of chemical constituents in Herbal extracts. Results confirmed the presence of alkaloid, tannins, terpenoids and saponins. Partition coefficient studies suggested the lipophilic behaviour of herbal extracts. Micromeritic studies of powdered herbal extracts suggested fair to good flowing property. Beer's law was obeyed for the method in the concentration range of 10-100 µg/ml. The entrapment efficiency of ethosomes depend on the concentration of lecithin (phospholipid) in the bilayer of vesicle. The entrapment efficiency of ethosomes depend on the concentration of soylecithin in the bilayer of the vesicle. Initially, with increasing lecithin concentration, the entrapment efficiency was increasing.[25] Visualization by SEM showed that ethosomes were spherical, that have an impact on the release of drugs. An increase in concentration of ethanol from (20-45%) generally decreases ethosomal vesicles size. A high concentration of ethanol, however, leads to lipid bilayer interdigestion and vesicle destabilization. Thus, our image confirmed the formation of ethosomes which were within the 1 µm size.[26] The FTIR spectra of all ethanolic extracts of *Curcuma longa* clearly exhibit identical peaks attributable to chemical components that are similar. However, closer examination reveals a little variance in peak intensity (absorbance) due to varying component concentrations in the ethanolic extract of *Curcuma longa*. *Curcuma longa*, which refer to phenolic chemicals responsible for Curcuma longa's yellow colour, are the major components of the ethanolic extract of *Curcuma longa*. The IR spectra of *Boswellia serrata* gum shows the wavenumbers (cm^{-1}) which confirms the presence of alcohol, amines, ketones, anhydrides and aromatic rings. The highest negative value of zeta potential was observed for the optimized formulation Ne9, which was considered favorable for the formulation stability and the enhancement of drug transdermal permeation due to the electrostatic repulsion between the same charge of the skin surface and meaning that the formulated ethosomes do not aggregate rapidly. The charge of vesicles is an important parameter that can influence both stability and skin vesicles interaction.[27] The release experiments clearly indicated sustained-release of herbal extracts from ethosomal formulations. The maximum release of Ne9 was found because of the higher drug content and entrapment efficiency of the formulation. The maximum release was also due to optimum phospholipid and ethanol concentration (i.e., 1.5 gm and 40 ml respectively), because at this concentration, molecule gets associated with the phospholipid bilayer resulting in better partitioning of the extracts, and resulted in higher drug release from the vesicles. Correlation coefficient study of different kinetic models showed that ethosomal formulations followed zero order and higuchi model which is best fit for transdermal delivery. Viscosity reduced as shear rate increased, according to rheological tests. As a result, it revealed the optimized formulations' usual pseudoplastic flow behavior. It was determined that the colloidal network structure aligned itself with the shear direction, lowering viscosity as the shear rate increased. Manufactured ethosomal gels had a good spreadability and be satisfied with optimal quality in topical application.[28] Extrudability and homogeneity were found to be in acceptable range. The permeation of Ne9 ethosomal gel was higher than that of Ne8 gel. It can be due to

the presence of high amount of ethanol i.e., 40 ml which contributes towards penetration and lead to release drug.[29] Propylene glycol present in ethosomal gel worked as a permeation enhancer and played a key role in improving the permeation. Therefore, the ethosomal gel had a good ability for transdermal permeation.[30] The presence of edge activator in the lipid bilayers of ethosomes caused the shape alteration. Plain gel has lesser penetration than ethosomal gel, which might be due to their stiff bilayers. Drying rate study justified that 2% w/v gel is suitable for further evaluation because of low rate of drying.[31]

CONCLUSION

Herbal ethosomes were prepared successfully by using different concentrations of phospholipid and ethanol as well as the incorporation of the ethosomes into carbopol 934 base gel to obtain ethosomal gel formulations. The prepared formulations were characterized for various properties. The compositions of ethosomes and gels were manipulated to investigate their effects on the characteristics of final formulations. It can serve as a useful vehicle for the delivery of herbal extracts through the affected part of the skin for extended period of time. This study also revealed that herbal ethosomal gel resides at targeted site for a relatively longer period of time with a zero-order release profile. It signifies the improved patient compliance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DN: Diabetic neuropathy; **TLC:** Thin layer chromatography; **SEM:** Scanning electron microscopy; **FTIR:** Fourier-transform infrared spectroscopy; **CMC:** Critical moisture content; **EMC:** Equilibrium moisture content.

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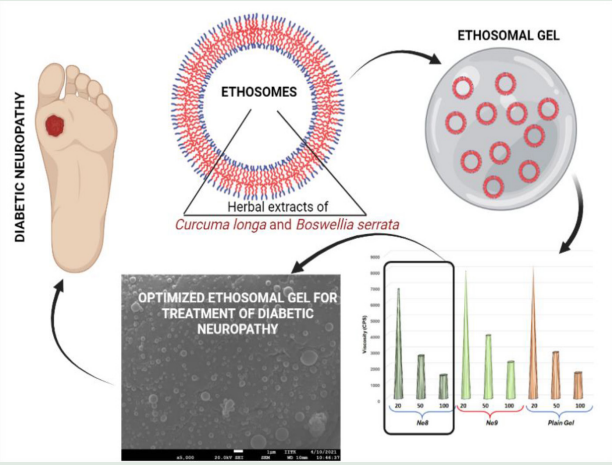
GRAPHICAL ABSTRACT



SUMMARY

Significant research is being done on herbal-based natural remedies to treat hyperglycemia and its negative effects in addition to commercially available formulations. The study's main goal was to use an ethosomal gel to increase the transdermal penetration of *Boswellia serrata* and *Curcuma longa* and to address the issues associated with limited transdermal drug delivery permeability. Cold-processed ethosomes containing *Boswellia serrata* and *Curcuma longa* were produced using varying concentrations of ethanol and soy lecithin, and they were enhanced based on *ex-vivo* tests, vesicular size, and entrapment effectiveness. Then a gel containing the improved ethosomal formulation was tested. *Curcuma longa* and *Boswellia serrata* ethosomal gels were used in ex-vivo experiments in comparison to plain gel. Vesicles with sizes ranging from 1 to 100 nm, a smooth, spherical surface, and a maximum entrapment effectiveness of Ne9 were created by the ethosomal system. In the gel, a modified ethosomal system (Ne9) was added and subsequently investigated. The results show that the transdermal administration of *Curcuma longa* and *Boswellia serrata* has a great deal of potential for the ethosomal gel.

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