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A STUDY ON ETHOSOMES AS MODE FOR TRANSDERMAL DELIVERY OF AN ANTIPSORIATIC DRUG

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ABSTRACT

The aim of the present research work is to prepare ethosome of Berberine chloride and to evaluate for vesicle shape, size, polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE), and in vitro permeation studies. Berberine Chloride loaded nanosized ethosomes were prepared using hot method with varied concentrations of ethanol and soyaphosphotidylcholine (SPC). The prepared ethosomes were characterized by size, entrapment efficiency (EE), FT-IR, in vitro release, kinetic studies of in vitro release profile and stability study. The selected ethosomal formulation was incorporated in carbapol 934P for gel formation, and subsequently, evaluated for their physicochemical properties. The sizes of ethosomes were found to be in the range of 94. 07-297.11nm while PDI ranges from 0.261 to 1.53 and ZP was between −20.6 to−40.1 mV. Morphology studies showed unilamellar structure under transmission electron microscope and optical microscopy. The EE of ethosomes was found to be in the range of 42.5% to 90.01%. It may be concluded that a Berberine chloride loaded ethosomal delivery system for herbal medications is a promising technique.

Keywords: ethosomes, soyaphosphatidylcholine, ethanols, Zeta Potential, unilamellar structure.

INTRODUCTION

Transdermal drug delivery systems are used to administer medications topically in the form of patches that release drugs for systemic effects at a predetermined and regulated rate. The practice of transdermal medicine delivery has been around for a while. Lotions and ointments administered topically was once the most popular approach for treating dermatological conditions[1]. Extended-release dosage forms, also known as TDDS, are able to maintain a constant level of medication in the bloodstream without causing first-pass metabolism. They can even assist with gastrointestinal problems brought on by medications and insufficient absorption[2]. The FDA approved the first transdermal patch, Transderm-Scop (scopolamine), for the treatment of motion sickness in 1981. The FDA next approved Transderm-Nitro (nitroglycerin), for the treatment of angina pectoris, in 1982[3]. Utilizing cutting-edge drug delivery technology, drugs are kept active at a set pace, their levels in the body are kept mostly constant (zero order kinetics), and unwanted side effects are prevented. To localize therapeutic

action in the damaged tissue or organ, it can also focus medicine distribution using carriers or chemical derivatization[4]. A significant challenge in developing transdermal drug delivery devices is the natural transport barrier of the skin, which prevents the transdermal distribution of therapeutic medications. We can break through the skin barrier using a variety of approaches. These include physical techniques (such as Iontophoresis, electroporation, sonophoresis, microniddle, magnetophoresis, thermophoresis, and skin abrasion) as well as chemical techniques (such as the use of penetration enhancers, prodrugs) and formulation techniques (such as liposomes, transferosomes, niosomes, ethosomes, virosomes, phytosomes, and cubosomes)[5].

Recently, ethosomes, a brand-new flexible vesicular technology, was found to be useful for topical/transdermal medication delivery. The design of carrier systems for the local and systemic delivery of hydrophilic and lipophilic medications is significantly impacted by this system's outstanding capacity to pass through human skin intact due to its high elasticity[6]. Additionally, the formulation's inclusion of ethanol encourages the development of lamellar-shaped vesicles, which have been demonstrated to enhance the solubility and trapping of a variety of medications, including minoxidil and testosterone[7]. Phospholipids and a high ethanol concentration in vesicular formulations have been linked to deeper penetration and distribution in the skin's lipid bilayers. Vesicles can include drugs that are both hydrophilic and lipophilic. Despite their conceptual complexity, ethosomal systems stand out for being simple to produce, safe, and effective—a combination that could significantly expand their application[8].

A common immune-mediated inflammatory skin condition called psoriasis is characterized by red plaques covered in thick scales. More than 80 years of psoriasis treatment have shown the efficacy of anthralin (1,8-dihydroxy-9-enthrone). Anthralin has a lot of unfavorable side effects despite being extremely effective in treating psoriasis. Because it stains skin, clothing, and any furniture it touches, using it is unpleasant[9]. Additionally, the isoquinoline alkaloid berberine has numerous therapeutic benefits, including those against viruses, bacteria, diarrhea, psoriasis, inflammation, and tumors[10,11].

Through the activation of adenosine monophosphate-activated protein kinase, stimulation of glycolysis, and inhibition of mitochondrial activity, berberine has several important effects on type 2 diabetes that enhance both lipid and glucose metabolism. There have also been reports of berberine’s effects on hypertension, congestive heart failure, cardiac arrhythmia, antibiotics, and wound healing. In addition to its positive effects, berberine has numerous drawbacks that have restricted its use, including as poor water solubility, minimal absorption, and low bioavailability. Nanotechnology has been seen as the primary approach of overcoming these restrictions[12-17].

MATERIAL AND METHODS

Materials

Berberine chloride was obtained as gift samples by Indian Pharmacopoeia Commission Ghaziabad. Soyaphosphatidyl choline was purchased from an Amitex Agro product Pvt Ltd. Nagpur. All other solvents and reagents used were of analytical grade.

Methods

By slightly altering the heating technique and utilizing ethanol (10–40%) and SPC (1-3%) in different concentrations, ethosomes were produced [Table 1]. By heating phospholipid in a water bath at 40°C until it becomes a colloidal solution, phospholipid is disseminated in water using this method. In a different vessel, ethanol and propylene glycol are mixed and heated to 40°C. Once both combinations have achieved 40˚C, the organic phase is added to the aqueous phase. The drug dissolves in either water or ethanol, depending on its hydrophilic/hydrophobic properties. The vesicle size of an ethosomal formulation can be decreased to the necessary amount using probing sonication or the extrusion method. The formulations were finally kept in the refrigerator[18.19].

Table 1: Composition of different Ethosomal formulations

Formulation Code	% SPC	Ethanol : water
F1	1	10:90
F2	1	20:80
F3	1	30:70
F4	1	40:60
F5	2	10:90
F6	2	20:80
F7	2	30:70
F8	2	40:60
F9	3	10:90
F10	3	20:80
F11	3	30:70
F12	3	40:60

SPC: Soyaphosphotidylcholine

Characterization

HPLC

Shimadzu class LC high-performance liquid chromatography system, equipped with FCV-10 ACVP pumps, DGU-14A degasser, thermostated CTO-10AVP column oven compartment, autosampler, and SPD-M10AVP diode array detector, was used to conduct the analysis. In intermediate precision experiments, the HPLC systems SPD-M10AVP and SIL-20AC were employed as equipments I and II. The column was a reverse-phase Zorbax ODS II (250 mm 4.6 mm, 5 m). With a mobile phase of buffer/acetonitrile, an injection volume of 10 l, and a flow rate of 1.0 ml/min, all analyses were carried out at a column temperature of 40± 1°C[20].

Determination of particle size, PDI, and zeta potential of Berberine chloride loaded ethosome

Using a Zetasizer Nano ZS90, the average particle size and zeta potential (ZP) of REB-SLNs were calculated (Malvern Instruments, UK). The produced REB-SLNs 100 l was diluted to 5 ml with double-distilled water in order to reach the appropriate kilo counts per second (KCPS) of 50-200 for measurements. All samples were double distilled water diluted to a suitable quantity prior to testing. Data were tested in triplicate and shown as mean±SD[21].

Entrapment efficiency

Separation of un-entrapped drug and evaluation of entrapment efficiency can be measured by ultracentrifugation. The entrapment efficiency was calculated using the following formula.

$$Entrapment\,efficiency = (T - C)/T * 100$$

Where, 'T' is the total amount of drug that is detected both in the supernatant layer and residence layer and 'C' is the amount of drug detected only in the supernatant[22].

Visualization of vesicles by transmission electron microscope (TEM) and optical microscope

For Transmission electron microscope (TEM), a drop of the material was applied to a copper grid coated with carbon, and after 15 minutes, it was negatively stained with a 1 percent phosphotungustic acid aqueous solution. The samples were inspected using a transmission electron microscope after the grid had fully dried by air (TEM, FEI-Philips Tecnai 10). Optical microscopy is also used to visualize vesicles[23].

Incorporation into hydrophilic gels

Among the 12 ethosomal batches, F 12 batch showed highest EE, optimum PDI and ZP which was further incorporated into carboxyvinyl polymer Carbomer (Carbopol 934P) gel formulations (Table 2). Smallest amount of water possible was used to absorb 1 percent carbopol w/v for an hour. The swelled polymer was combined with 20 ml of ethosomal suspensions containing 100 mg of berberine hydrochloride while being continuously stirred at 30 ˚C to produce uniform gels. Triethanolamine was then used to bring the pH to a neutral range, and the mixture was slowly

mixed until a clear, translucent gel was produced. The final ethosomal formulation was continuously stirred for 30 minutes at room temperature. The stability, pH, viscosity, vesicle size, shape, surface morphology, entrapment efficiency, and in vitro drug penetration of the formulations were assessed while they were refrigerated[24].

Viscosity and pH measurement

Viscosity of ethosomal gel formulation was measured using a Brookfield Viscometer (Model No DV-III ULTRA) using spindle no 06 at 100 rpm, and pH measurements of the formulations were done using a digital pH meter (RI-152-R)[23].

Spreading diameter

By measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm 20 cm) after 1 min, the spreadability of gel formulation was ascertained. The upper plate was given a standard weight of 125 g[24].

Drug content of the formed gels

About 500 mg of gel was taken and dissolved in 50 ml of pH 7.4 PBS. The solution was then passed through the filter paper, and 50 µl of the filtrate was withdrawn. The filtrate was diluted by adding 3.5 ml of distilled water, and the drug content was measured spectrophotometrically at 346nm against corresponding gel concentration[24].

In vitro diffusion studies

For ethosomal dispersion (F 12), ethosomal gel formulation (GELF12), and conventional gel formulation utilizing dialysis membrane, an in vitro diffusion research was carried out (Hi media). Before beginning the permeation investigation, it was immersed in PBS (7.3) for 6 hours to reach saturation. . It was then mounted between the donor and receptor compartments of the Franz diffusion cell (fabricated with glass, the surface area available for diffusion was 2.54 cm2). By inserting the necessary sample into the donor cell compartment, the release rate of berberine hydrochloride was evaluated. The donor chamber was paraffin-covered to guard against contamination and evaporation. PBS (7.4) was used to fill the receptor chamber, which was kept at 37°C. At intervals of 0.5, 1, 2, 4, 8 and 24 h, 1 ml of the receptor phase solution was removed, and the same volume of fresh medium was then put back into the chamber. A UV spectrophotometer (Shimadzu Model No. 1800) set to 346 nm was used for the quantification. Transdermal flux (J) was determined from the slope of the linear part of a cumulative amount of drug penetrated per unit area against time graph[25].

Permeation data analysis

The results from the in vitro drug release investigation were fitted in various kinetic models to analyze the release rate profile. The cumulative percent of drug remaining versus time is represented by zero order, the log cumulative percent of drug remaining is represented by first order, the cumulative percent of drug remaining versus the square root of time is represented by Higuchi's model, the cumulative percent of drug remaining versus Hixson Crowell cube root

model, and the cumulative percent of drug released is represented by the KorsmeyerPeppas model[23].

Statistical analysis

Data are expressed as mean ± standard error of mean. Differences in the in vitro release profile of prepared formulations were tested for significance using independent t-test using SPSS-12. 0. The difference was considered significant when $P < 0.05$. Graphs were prepared using GraphPad Prism 3 (Graph Pad Software, Inc)[23].

Stability study

For the stability research, optimized ethosomal formulations were chosen. Formulas were kept in refrigerators at 4°, 8°, and room temperature. At various time intervals, the percentage of drug entrapment was calculated (1, 15, 30 and 45 d).

RESULT AND DISCUSSION

In this study, we evaluated ethosomes as carriers for the topical application of Berberine Chloride. Twelve ethosome formulations were prepared using different amount of SPC and ethanol as presented in Table 1.Ethosomes can penetrate the stratum corneum to the deep layers, as ethosomes contain high alcohol content. It has been revealed that the higher alcohol content and synergistic effects of phospholipids in ethosomes enables the entrapped drug to reach deeper epidermal layer. The above finding is supported by a study that found that trihexyphenidyl hydrochloride ethosomes made of phospholipid, ethanol, and water have an 87-fold increase in steady-state transdermal rate when compared to typical liposomes. The amount of drug entrapped inside the vesicles is indicated by entrapment efficiency, and according to Table 2 findings, F12 ethosomes exhibited the highest level of entrapment efficiency. Particle size is considered as a major parameter in the selection of optimum formulations. However, once the ethosomes underwent probe sonication the diameters of all ethosomes in the formulations were approximately 250 nm. Therefore, we used entrapment efficiency and skin deposition as the major parameter which determined the selection of the next set of formulation(s) for testing[26,33,34].

In the present study, the HPLC characterization of Berberine Chloride was carried out. The purity obtained for drug sample was 97.03 and the purity of the standard was 97.50 and considered as a positive result for Berberine Chloride (Figure 1).

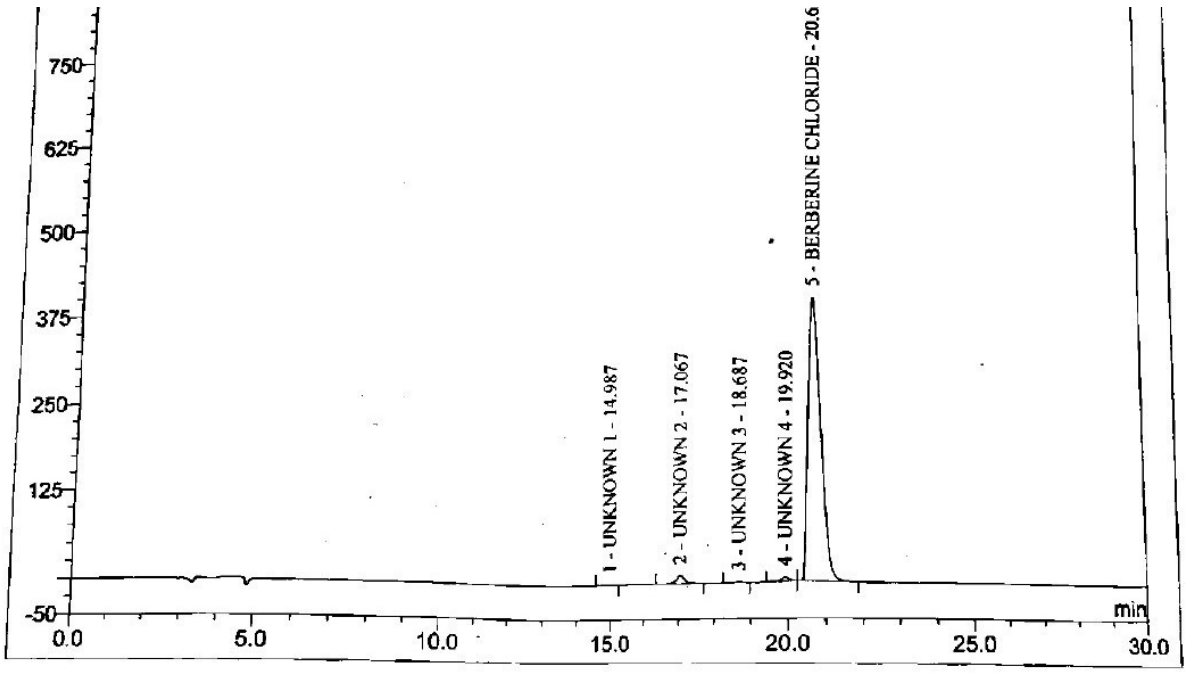

Fig 1: Berberine Chloride Chromatogram in HPLC

Major peaks were seen in the IR spectra for the drug excipient compatibility investigation at wavelengths of 3382.4 cm^{-1}, 2926.1 cm^{-1}, 1646.8 cm^{-1}, 1423 cm^{-1}, 1367 cm^{-1}, and 1042 cm^{-1}. The corresponding peaks were likewise found in the drug excipient mixture with modest shifting. A unique peak at 1738 cm^{-1} was seen in addition to these peaks, confirming the presence of phosphate groups in soya lecithin. The data clearly show that the distinctive peaks of the extract were unaffected by the addition of soy lecithin, suggesting that the two are complementary substances (Figures 2 and 3).

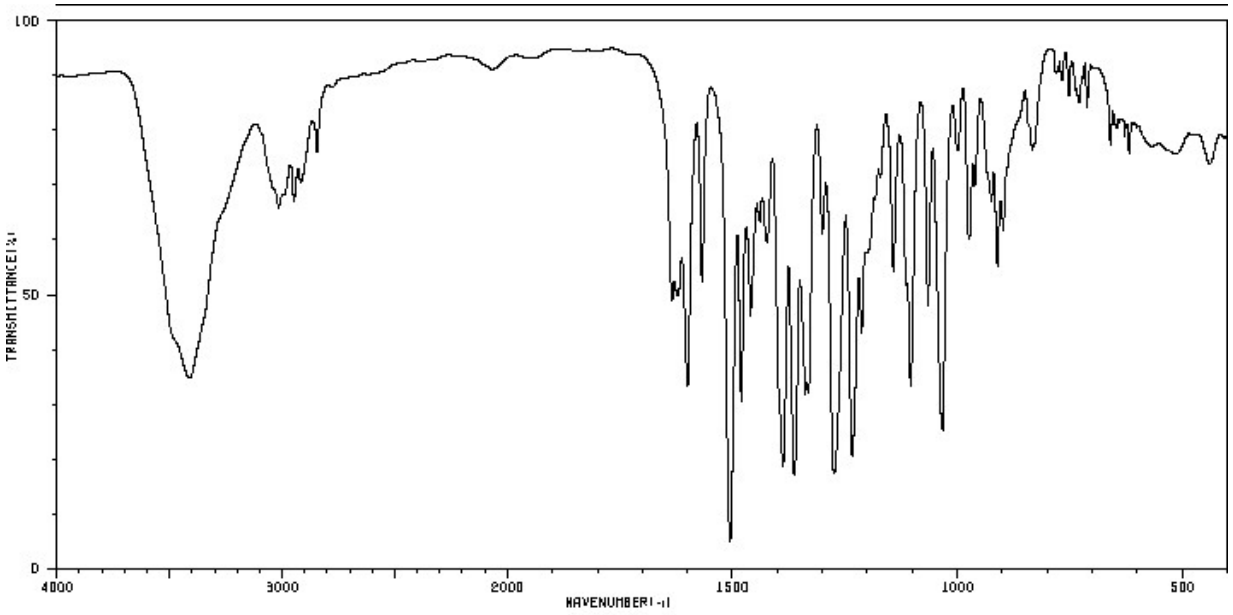

Fig2: Infrared spectrum of Berberine chloride

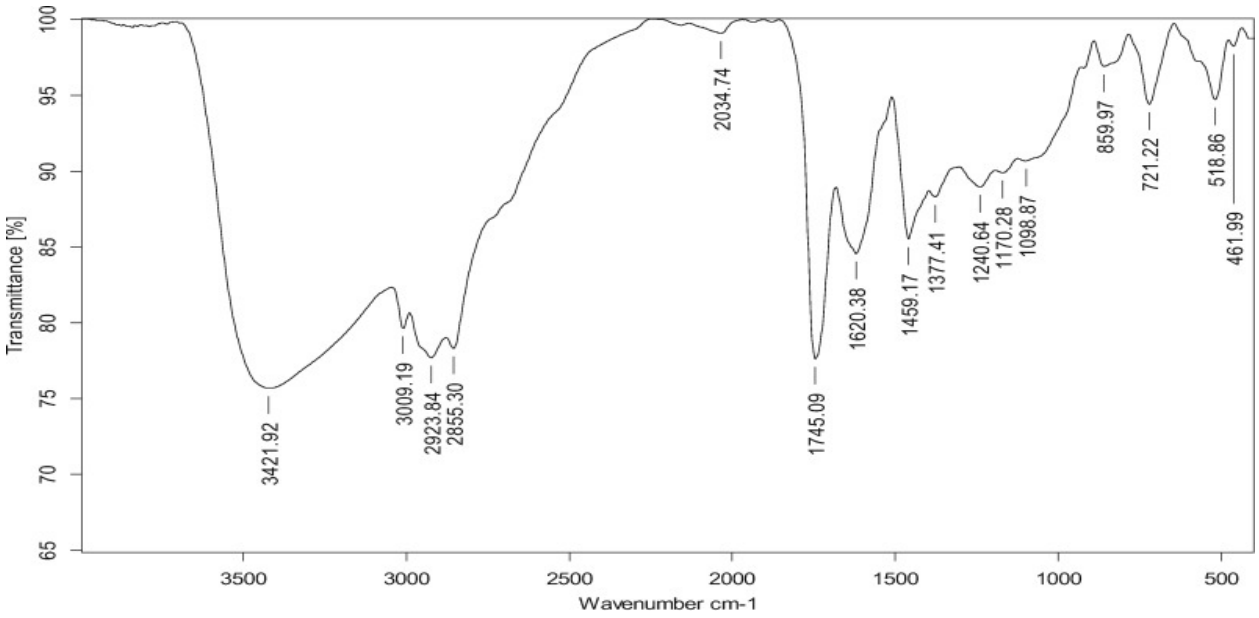

Fig 3: Infrared spectrum of F12 ethosomal formulation

Overall, vesicular size and morphology largely control how well transdermal drug delivery systems operate.

A 100 X optical microscope was used for optical microscopy. In (Figure 4) ethosomal vesicles transmission electron micrograph revealed that they had a spherical form and a unilamellar structure (Figure 5). The photographs all show smooth surfaces.

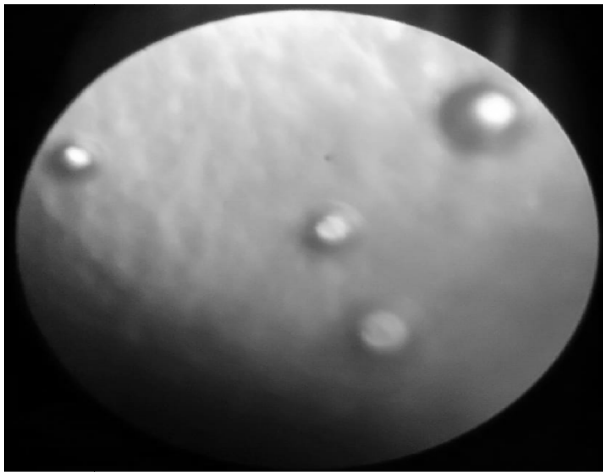

Fig 4: Optical Microscopy image of ethosomal formulation (F12) (100X magnification)

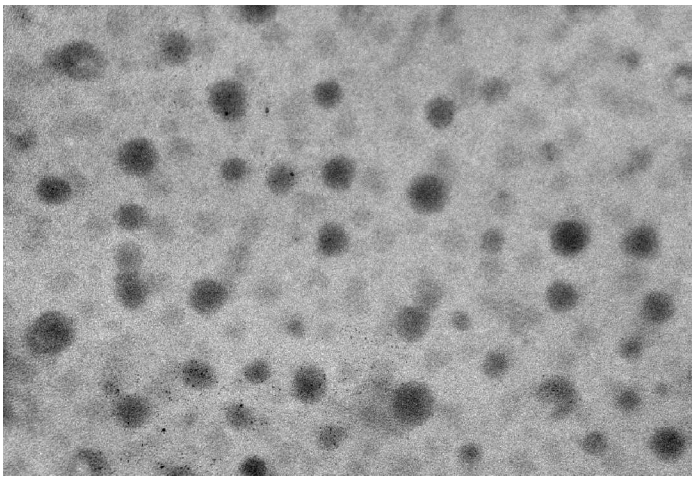

Fig 5: Transmission electron microscopy image of ethosomal formulation (F12)

According to certain reports, vesicles with a size of less than 300 nm can convey their contents into the deeper layers of skin [27, 28]. Size study of the formulation revealed that it ranged from 94.07 to 297.11 nm depending on the proportion of SPC and ethanol. The results of vesicle size are presented in table 2.

Increasing concentrations of ethanol (10%, 20%, 30%, and 40%) and SPC concentration resulted in ethosomal dispersions (1 percent, 2 percent, and 3 percent). The size of the vesicles in the research dropped from 204.10 to 94.07 nm by increasing the percentage of ethanol from 10% to 40% while retaining the concentration of SPC at 1%. Similar to this, the ethanol concentration was raised from 10% to 40%, while the SPC concentration was measured at 2% and 3%. Vesicle size was discovered to have shrunk from 260.06 to 204.10 and 297.11 to 175.2 nm, respectively. The results are presented in table 2.

The data show that the size of the vesicles increased with increasing concentrations of SPC from 1 to 3 percent, but alcohol concentration had an opposite effect, producing smaller vesicles at higher concentrations. This observation supports the results, which show that increasing ethanol

concentrations causes vesicle sizes to shrink because they provide a surface negative net charge to the vesicular systems by changing certain surface properties [29, 30]. Polydispersity index (PDI) was considered for evaluation of homogeneity of prepared ethosomes on the basis of their vesicle size distribution. The PDI value lay between 0.104 and 1.53, inferring that all the batches showed a narrow distribution except batches F1, F2 and F3.

Zeta Potential is a crucial variable that impacts stability. Due to the formulation's net charge from the lipid composition, it was discovered that every ethosomal formulation had a negative ZP (-20.6 to 40.1 mV). Drug percutaneous penetration is improved thanks to the negative Zeta Potential.

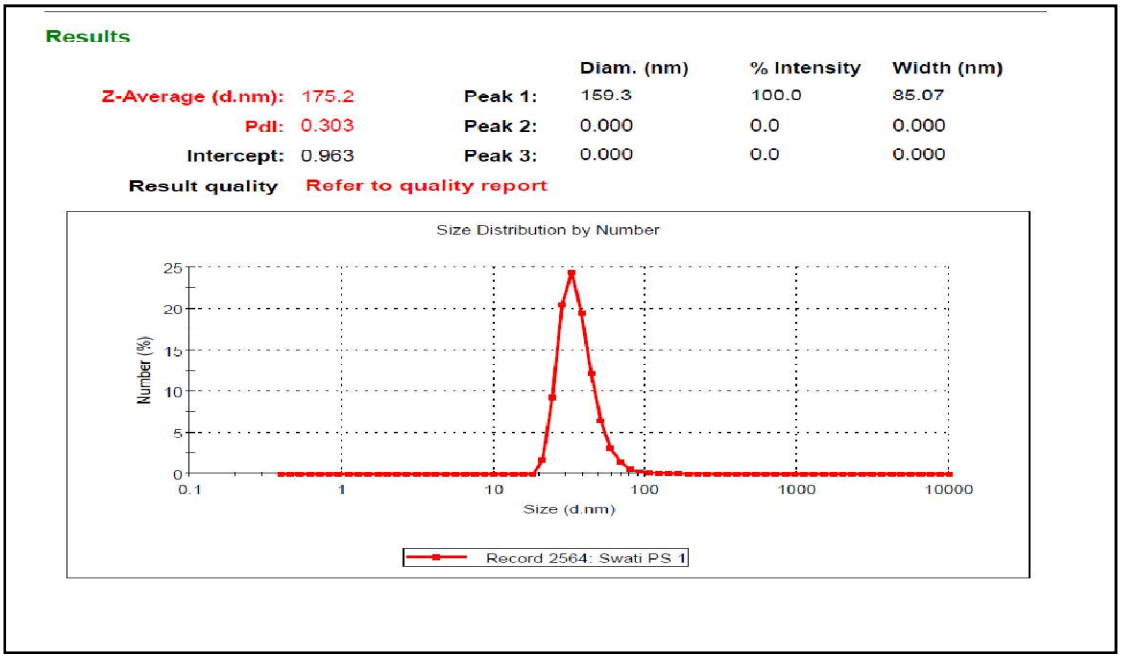

Fig 6: Size and size distribution F12 ethosomal formulation

The drug carrying capacity of the ethosomal system, which is measured in terms of EE, directly affects its delivery potential. All of the formulations ethosome expression levels (EE) were calculated. The amount of SPC and ethanol had a positive impact on the herbal extract's ability to become trapped inside lipid vesicles. The ethosomes EE values fell between the ranges of 42.5 and 90.01 percent. The ethosome system's ethanol concentration should be kept below 45 percent and shouldn't be too high. Only ethanol concentrations up to 40% were examined because higher ethanol concentrations cause drug leakage from the lipid bilayer, which reduces EE[26,31].

Ethosomal formulation made with 3 percent SPC and three different concentrations of ethanol (20%, 30%, and 40%) shown 85.0%, 85.71%, and 90.01% EE, respectively. The results are presented in table 2. . A change in EE was also seen while using different SPC concentrations (1 percent, 2 percent, and 3 percent) with a constant level of ethanol (30 percent) (SPC 3 percent). Thus, it may be concluded that EE is reliant on the levels of both ethanol and SPC. Higher EE

with more ethanol may be caused by Berberine chloride's higher solubility in the ethanol present in the ethosomal core. This is in accordance with the previous finding by Paolino et al., 2012[21].

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

Formulation Code	Entrapment Efficiency (%)	Vesicle size (nm)	PDI	Zeta Potential (mV)
F1	42.5±1.82	225.12±12	0.847±0.052	-38.0
F2	54.2±1.12	137.04±14	1.53±0.038	-36.2
F3	65.5±0.82	108.16±13	0.956±0.042	-31.2
F4	48.5±0.82	94.07±08	0.391±0.052	-25.3
F5	72.5±2.02	260.06±15	0.412±0.016	-38.2
F6	82.0±1.22	245.15±19	0.293±0.007	-35.2
F7	78.14±0.80	215.12±12	0.467±0.052	-33.3
F8	77.5±0.82	204.10±10	0.472±0.052	-36.2
F9	83.0±0.84	297.11±11	0.375±0.003	-40.1
F10	81.01±2.11	281.16±13	0.261±0.013	-20.6
F11	85.71±0.83	265.34±14	0.392±0.013	-28.9
F12	90.01±1.16	175.2±11	0.303±0.052	-31.1

Data represents mean±SD, n = 3

For in vitro diffusion investigations (Figure 1a and b), batch F12 was chosen due to its small vesicle size, consistent size distribution, and greater EE. It was then added to a gel formulation (GELF12) for in vivo studies. One crucial trait of ethosomes is the sustained release property. Calculations were made to determine the total amount of drug release from in vitro diffusion trials for the formulations of standard gel, GELF 12, and F12. For Batch F12, GELF 12, and standard gel formulation, the percentage cumulative amount of drug release in 24 hours was found to be 78.21 percent, 74.34 percent, and 30.09 percent, respectively (Figure 7).

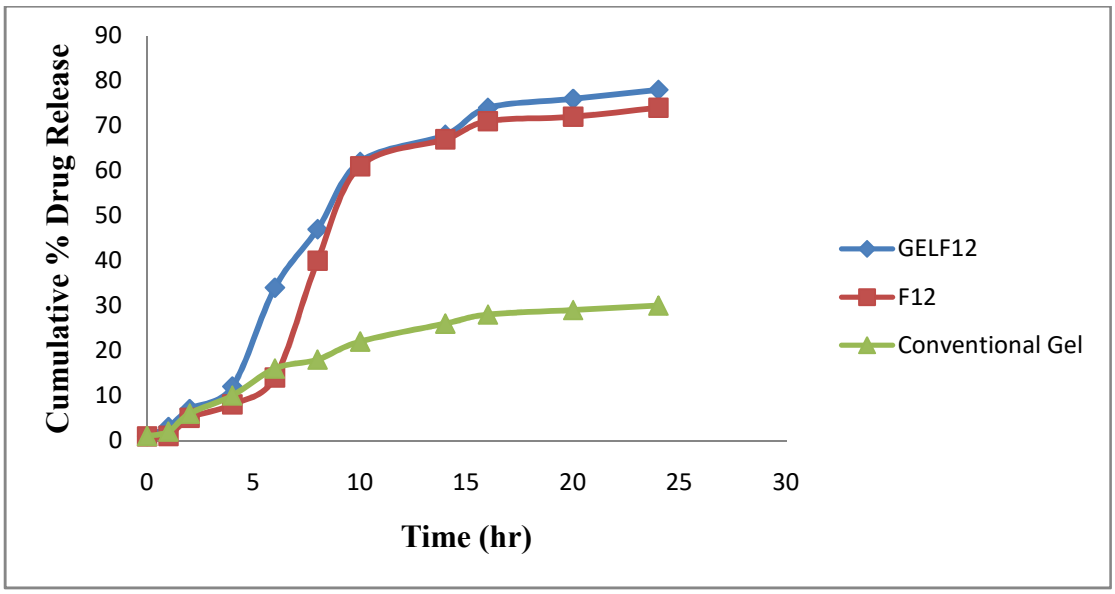

Fig 7: *In Vitro* release profile of GELF 12, F12 and conventional gel formulation. Data shown are means±SEM (n= 3)

Among them, Batch GEL F12's percent medication released was significantly higher than that of traditional gel formulation ($P < 0.05$). Ethosomes percent cumulative amount of medication release is also noticeably larger than that of traditional commercial formulations ($P < 0.05$). This might be because ethosomal formulations have ethanol in them while regular gel doesn't contain any ethanol at all. The soft, flexible properties of ethanol provide the vesicles the ability to penetrate deeper layers of skin more easily. Additionally, this is consistent with earlier research by Chourasia et al[30].

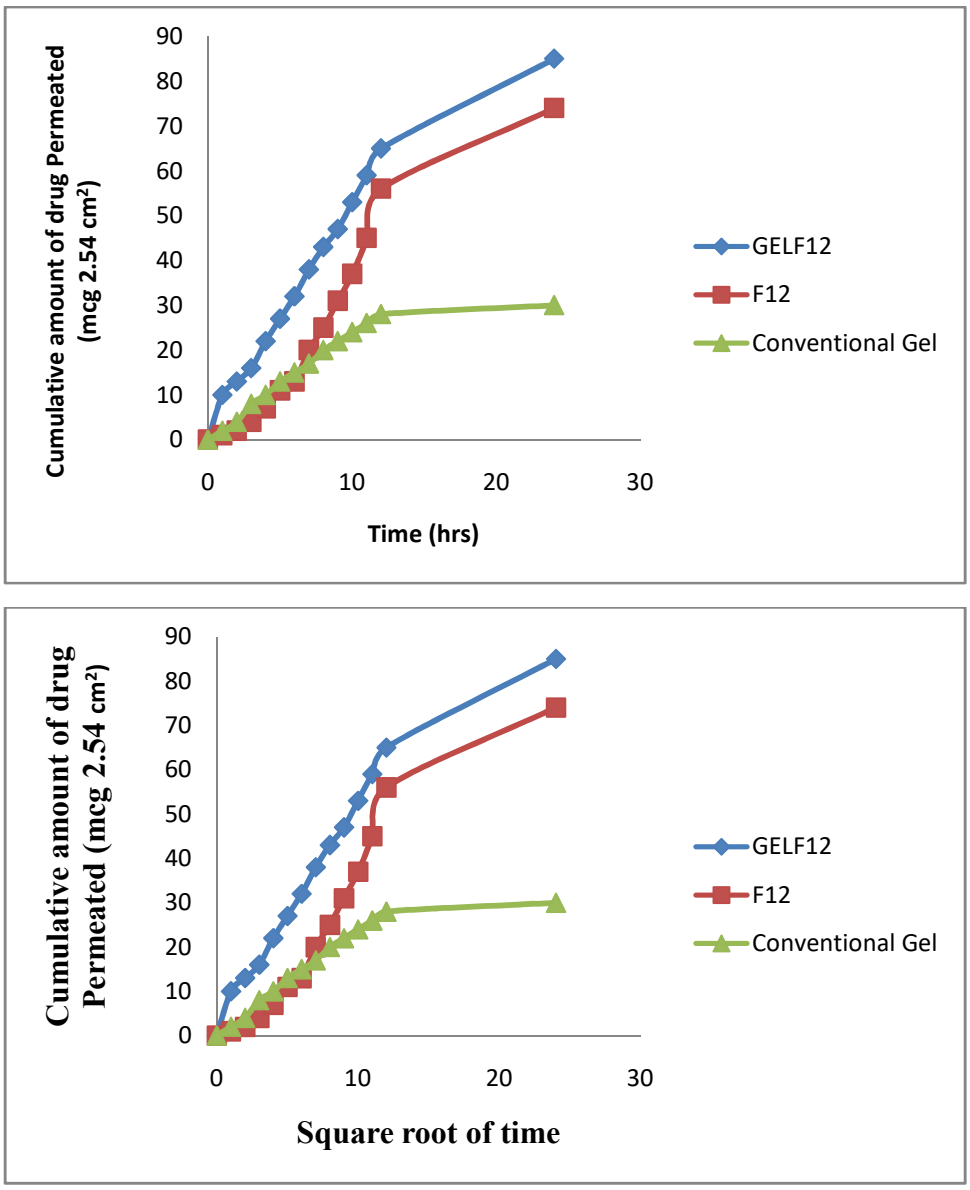

Fig 8: (a) Comparability graph of in-vitro permeation of Berberine Chloride from various formulations through 2.54 cm^2 values represent mean ± SEM (n=3), (b) comparative graph of cumulative amount of drug permeated versus the square root of time. Results are expressed as means±SEM (n= 3)

Transdermal flux (J) was estimated from the slope of the linear component of a graph of the cumulative amount of medication penetrated per unit area vs. time (Figure 8a). The results of the investigation of transdermal flux of the identical formulations were similar to those of cumulative percent drug release (Figure 8b), ranging from 66.69 to 163.5 g/cm2/h (traditional gel) (GEL F12). The findings show that ethosomal gel had a flow that was 2.45 times more than that of ordinary gel.

To get the release rate profile, the data from the in vitro release investigation was fitted to various kinetic equations. The Higuchi kinetic model was determined to have the greatest match for batches F12 and GELF12, with r^2 values of 0.880 for F12 and 0.931 for GELF12. This suggests a gradual release through the diffusion mechanism, as stated by Higuchi.

The physical characteristics, pH, spreadability, viscosity, and medication content of the produced gels were assessed. The gels were discovered to be uniform, smooth, yellowish white in colour, with a pH that was within the typical range of skin, easily spreadable, and with a viscosity of 4521 to 4836 cps.

Table 3: Evaluation of physicochemical properties of gel formulations

Formulation Code	Colour	Homogeneity	Texture	pH	Viscosity (Centipoise)	Spreading Diameter after 1 min (mm)	Drug Content (%)
GELF-12	Yellowish Brown	Homogenous	Smooth	6.78±0.12	45215±1.21	55±1.5	46.47±0.80

Data represents mean±SD, n = 3

The optimized formulation (F12), vesicular size, shape, and entrapment efficiency data showed that the formulation was equally stable at room temperature and under refrigeration (Table 4).

Table 4: Results of stability studies of optimized formulations (F12)

Temp	Initial		After two month	
	EE	**Vesicle Size (nm)**	**EE**	**Vesicle size (nm)**
2˚C	78.71±1.48	497.2	78.501±1.23	500.1
25˚C	78.71±1.48	497.2	78.53±1.56	502.4

Data represents mean±SD, n = 3

CONCLUSION

In comparison to the traditional formulation of Berberine Chloride, the developed new ethosomal system, including Berberine Chloride demonstrated an improved penetration profile. We come to the conclusion that transdermal administration of Berberine chloride via the ethosomal system may be a more effective strategy for treating dermatological conditions. To investigate the therapeutic potential of this extract for treating dermatological illnesses, however, additional

biological and clinical research must be conducted. This will aid in the creation of secure and effective herbal formulations.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

No conflict of interest was declared by the authors.

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