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Research Article

Formulation and Evaluation of Ethosomal Cream containing Resveratrol for the Treatment of Contact Dermatitis

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Abstract

Objective: The aim of this study was to formulate and evaluate ethosomal cream containing resveratrol for the treatment of contact dermatitis.

Method: Resveratrol-loaded ethosome was prepared using cold method. The prepared ethosomes were assessed for entrapment efficiency (%), TEM analysis and sedimentation rate to determine the optimal formulation for loading into cream base. The selected formulation was further investigated for particle size distribution and zeta potential. A cream base was formulated using fusion and trituration method and selected for loading with the ethosomal suspension, meeting specification suitable for topical delivery. Ethosomal cream formulations were evaluated for drug content, ex -vitro diffusion by using goat skin, skin retention, microbiological study and stability study.

Result and Discussion: The formulation E3 was selected for loading in the optimized cream base (F4) to obtain a topical cream containing ethosomes of resveratrol. The release study reveals that release was controlled for the 24 hours. The drug release in buffer was less than 17% from all the formulations after 24 hours. The drug content of resveratrol in goat skin and cream remained in donor compartment indicates that the drug retention in skin was high in all the formulations, which is desirable for the topical formulations. The best possible release kinetics of the formulation loaded with resveratrol containing ethosomes was found to be zero order. The 'n' value from Peppas's model indicated that the drug release was by Fickian diffusion. Stability study indicates that all the cream formulations were stable after a time period of one month.

Conclusion: The present study conclusively supported that ethosomal cream containing resveratrol to be an advantageous topical drug delivery system in treatment of ACD. However detail in- vivo study should be conducted in future to be justify the in- vitro study.

Keywords: Resveratrol, Ethosome, Cream, Contact dermatitis.

1. INTRODUCTION

Allergic contact dermatitis is hypersensitivity reaction which includes symptoms like erythema, edema, vesicles, and intense pruritus [1]. When the immune system encounters a contact allergen, it triggers an innate immune response. Langerhans and dermal dendritic cells take up the allergen and migrate to nearby lymph nodes, where they activate antigen-specific T helper (TH) cells such as TH1, TH2, TH17, and regulatory T [Treg] cells. These activated TH cells then multiply and enter the bloodstream. Upon encountering the allergens at the exposure site, TH cells induce inflammation by releasing cytokines like IL- 4, IL- 5, IL-8, IL- 13 and IL- 10 [2,3].

The main approach of managing ACD involves educating patients about allergen avoidance to prevent reactions. Polidocanol is applied topically as a moisturizing agent in dermatitis treatment, but it may cause mild itching and burning sensation as a side effect [4]. For localized acute allergic contact dermatitis, triamcinolone 0.1% or clobetasol 0.05% are effective topical steroids. Thinner skin areas may benefit from desonide ointment, lower-potency steroids [5]. The chronic phase is managed by moisturizing creams for skin dryness in addition to topical steroids [6]. Class II and III topical corticosteroids are recommended for inflamed skin, but long-term use may lead to skin atrophy. Alternatively topical approaches like calcineurin inhibitors and phototherapy are less recommended in ACD [7].

Phytoconstituents from Artemisia vestita, Panax ginseng and Sophora flavescens are used in traditional systems like Ayurveda and Chinese medicine for treating ACD [8]. Resveratrol a polyphenol found in various plants grapes, peanuts and in mulberries is utilized in traditional system like Ayurveda and Chinese medicine for treating ACD [9]. Resveratrol has low solubility in water and is lipophilic [10]. Systemic half-life of resveratrol ranges between 2-2.5 hours, with an oral bioavailability of approximately 75%. However, the oral route lacks the advantage of local delivery to allergic skin compared to the topical route [11]. Resveratrol primarily inhibit cytokine release, thereby halting T cell activation and subsequently reducing inflammation [12].

Topical drug delivery for ACD offer targeted treatment affected skin, increased bioavailability by bypassing first pass metabolism, prompt cessation of treatment for adverse effects,

and importantly, enhances patient compliance by avoiding corticosteroid-related side effects [13]. Ethosomes, as a novel form of liposome, serve as an effective carrier for topical or transdermal administration. They consist of high concentrations of phospholipids and alcohol such as ethanol and isopropyl alcohol, and can encapsulate hydrophilic, lipophilic, or ampiphilic drug molecules. With high deformability and entrapment efficiency, ethosomes penetrate the skin efficiently, facilitating drug delivery into deeper skin layers and even the bloodstream compared to other liposomes [14]. So, in the proposed research work ethosomal cream containing resveratrol will be formulated and investigated for its potential in the management of ACD.

2. MATERIALS AND METHOD

Resveratrol was obtained as a complimentary sample from Yucca Enterprises Mumbai, India. Cetyl alcohol, stearic acid, triethanolamine, methyl paraben, cholesterol, phosphatidylcholine were obtained from CDH Fine Chemical, New Delhi. All other solvent reagents utilized were of analytical grade and used as received.

2.1 Preformulation studies: Preformulation studies are carried out in order to determine the physicochemical properties of drug which helps in the design of a safe, efficacious and stable dosage form. The resveratrol was underwent characterization for its organoleptic properties, such as color and odor, and was then compared with the standard outlined in the official monograph**.** Melting point of resveratrol was determined by using digital melting point apparatus**.**

2.1.2 Determination of absorption maxima (λ_{max}) of Resveratrol

An absorption maxima(λ_{max}) of resveratrol was determined in ethanol by scanning the 0.5 µg/ml solution in range of 200-400 nm using UV-Visible spectrophotometer (LABINDIA 3000+**).**

An absorption maxima (λ_{max}) of Resveratrol was determined in pH 6.8 phosphate buffer by scanning the 1 µg/ml solution in range of 200-400 nm using UV-Visible spectrophotometer [15, 16].

2.1.3 Preparation of calibration curve: An accurately weighed 100 mg of resveratrol was dissolved in small volume of ethanol in the 100 ml volumetric flask and final volume was adjusted with the same solvent. A series of standard solutions in Beer's - Lambert range of concentrations 1 µg/ml, 1.5 µg/ml, 2 µg/ml, 2.5 µg/ml, 3 µg/ml, 3.5 µg/ml, 4 µg/ml and 5 µg/ml of resveratrol were prepared and absorbance was measured at 307 nm against blank using an UV-Visible spectrophotometer.

2.1.4 Determination of drug solubility: The solubility of resveratrol was determined in water, methanol, ethanol, pH 6.8 phosphate buffer. In the known volume (10 ml) of solvent in different volumetric flasks, small increments of drugs were added with shaking until the saturation then drug solution was equilibrated at 32 ± 0.5ºC for 72 hours, solutions were filtered using whatmann filter paper. The absorbance of filtrate was measured at 307 nm for resveratrol, respectively using UV-Visible spectrophotometer [17].

2.1.5 Fourier transformed infrared (FTIR) study of pure drug: FTIR spectra of pure drug were obtained using KBr press pellet method. Resveratrol, in its pure form, was evenly blended with dry KBr powder at a ratio of 1: 100. The resulting mixture was then compressed into transparent discs under high pressure using special dies [18].

2.1.6 Drug –excipients compatibility study: Compatibility screening of resveratrol with different excipients like cetyl alcohol, stearic acid, methyl paraben, propyl paraben, triethanolamine, glycerin, propylene glycol, cholesterol, and phosphatidylcholine was investigated by recording the FTIR spectra of different excipients with drugs by KBr disc method and comparing the important peak positions with FTIR spectrum of pure drug.

2.2 Formulation and evaluation of ethosome of resveratrol

2.2.1 Formulation of ethosomes: Resveratrol loaded ethosomes (100ml) was prepared using cold method. Different concentration of phosphatidylcholine as lipid, cholesterol as stabilizer, ethanol as penetration enhancer and solvent were selected on the basis of literature survey shown in Table No. **1**. Phosphatidylcholine was dissolved in ethanol using mechanical stirrer (Remi, Mumbai) at 800 rpm. Temperature was maintained at 30°C by placing beaker on heating mantle. Cholesterol and resveratrol were added in the dispersed phosphatidylcholine, followed by slow addition of water under continuous stirring. Prepared formulations of ethosomes were stored in refrigerator for further evaluation [19, 20].

Table 1: Formulation of ethosomes of resveratrol.

S.NO	Formulation Code	Phosphatidyl choline (%)	Ethanol (%)	Drug (mg)	Cholesterol (%)	Water (%)
1	E1	2	30	100	0.25	70
2	E2	2	30	100	0.5	70
3	E3	3	30	100	0.25	70
4	E4	3	30	100	0.5	70

2.2.2 Evaluation of resveratrol ethosome: Resveratrol ethosomal formulations were evaluated for optical microscopy, transmission electron microscopy (TEM), entrapment efficiency, sedimentation and in vitro diffusion study using dialysis membrane. The optimized formulation on the basis of morphology, entrapment efficiency and sedimentation was further evaluated for particle size and zeta potential [21, 22].

2.2.2.1 Optical microscopy: Morphology of all formulations was determined using optical microscopy at 45X magnification with a digital SLR camera.

2.2.2.2 Transmission electron microscopy: The optimize formulations morphology was studied using TEM **(Philips EM420 TEM)**. Samples were prepared using drop-cast techniques on a carbon-coated copper grid and imaged at 100 kV with various magnifications.

2.2.2.3 Determination of sedimentation: Ethosomal dispersions were physically observed for turbidity and sedimentation at 0, 15, 30, 45, 60, and 90 days interval. The sedimentation rate results were reported based on bottom-view observations of the containers having ethosomes.

2.2.2.4 Entrapment efficiency: Entrapment efficiency of the ethosomal formulations were determined using indirect method. Formulations were centrifuged at 5,000 rpm and 4°C for 30 minutes to separate unentrapped drug. The supernatant was collected, and vesicles were washed with double distilled water and centrifuged again. Combined supernatants were diluted with, ethanol, filtered and analyzed by the UV- visible spectrophotometer to quantify resveratrol.

Drug entrapment efficiency was measured by the following equation:

$$Entrapment\ efficiency\ (\%) = [(E1 - E2) \div E1] \times 100$$

Where, E1: initial amount of drug, E2: quantified drug in the supernatant

2.2.2.5 In vitro diffusion study using dialysis membrane: In vitro permeation studies were conducted using Franz diffusion cells with Hi media dialysis membrane. Formulation equivalent to 1 mg of drug was applied to donor compartment contained 30 ml of pH 6.8 phosphate buffer at 37 °C. Samples were withdrawn at intervals and replaced with fresh medium [23, 24].

2.2.2.6 Particle size and Zeta potential: The particle size and zeta potential of the optimized formulation were determined using **Delsa ™ Nano**. The performance of ethosomes system depends on the surface charge of the vesicles. The value of Zeta potential indicates the stability of vesicular formulations by assessing the degree of repulsion between similarly charged particles in the dispersion system. It is measured by observing the response of charged particles to an electric field using zeta sizer [21, 22].

2.3 Formulation and evaluation of cream

2.3.1 Formulation of cream: The base cream (60 g) was prepared through fusion and trituration methods. The composition of the cream base is shown in Table No. **2**. The oil phase containing cetyl alcohol and stearic acid was melted at 65°C, while the aqueous phase, containing propylene glycol, glycerin and triethanolamine was also maintained at 65°C. Methyl paraben and propyl paraben (1:0.5 ratios) were added as preservative [25].

Table 2: Formula for cream base.

S.no	Ingredients (%w/w)	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Cetyl alcohol	2	2	2	2.5	2.5	2.5	3	3	3
2	Stearic acid	9	10	11	9	10	11	9	10	11
3	Propylene glycol	5	5	5	5	5	5	5	5	5
4	Glycerin	5	5	5	5	5	5	5	5	5
5	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
7	Triethanolamine	2	2	2	2	2	2	2	2	2
8	Double distilled water	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100	Qs to 100

2.3.2 Evaluation of cream: Cream formulations were evaluated for organoleptic and physicochemical properties [26, 27].

2.3.2.1 Physical appearance: The cream base underwent visual evaluation for color, homogeneity, smoothness and consistency.

2.3.2.2 Grittiness: To assess grittiness, a small portion of the cream base was placed between two grease-free glass slides and examined under diffused light to detect any foreign particles.

2.3.2.3 Determination of pH: To determine the pH of cream base sample (5g±0.1g) was placed in a 100 ml beaker. Distilled water (45 ml) was added, and the mixture was heated to 45°C while constantly stirring for 15 minute using a glass rod on a heating mantle. After filtration, the pH was measured at 27°C using digital pH meter.

2.3.2.4 Spreadability: To evaluate spreadibility, samples were weighed and placed on a custom-made spreadability instrument consisting of glass plates. The lower plate was fixed to a wooden platform, and a second glass plate (10×6 cm) with a weight of 50g was placed on the top for pulling towards upper plates, to slide from one designated point to another 7 cm away was recorded. The spreadibility (S) was calculated using the equation:

$$S = M \times L/T$$

Where, 'S' is the spreadability of the cream formulation, 'M' is the weight (g) tied on the upper plate, 'L' is the length (cm) of the glass plates, and 'T' is the time taken (s) for the plates to slide the entire length.

2.3.2.5 Viscosity: To assess viscosity (in cps), the cream base formulation was examined for viscosity using Brookfield rheometer (Cone and plate) (R/S Plus). One gram of sample, kept at maintained at 25±1°C, was placed on the plate, and the cone was rotated at 10 rpm.

2.3.2.6 Thermal Stability: The cream formulation was spread along the internal wall of a 100 ml glass beaker using a spatula. The beaker was then placed in a stability chamber (Thermolab scientific equipment, Mumbai) maintained at 60-70 % RH and 45 ± 1°C for 48 hours. Following this period

formulations were inspected for any separation of the oil phase.

2.3.3.7 Determination of total fatty content: Two gram sample is refluxed with 25 ml of hydrochloric acid, and then extracted with 50 ml of ethyl ether. After washing to remove acid, the solution is filtered over sodium sulphate and dried at 60°C ± 2°C until a constant mass. The percentage of total fatty mass is calculated using formula:

$$\text{Fatty acid (\%)} = 100\times M1/M2$$

Where,

M1= Mass obtained from residue, M2 = Total mass.

2.3.3.8 Determination of residue: Five gram of the formulation is dried at 105±1°C until a constant mass is achieved, then weighed. The residue content is calculated using the formula:

$$\text{Residue (percent by mass)} = 100\ (M_1/M_2)$$

Where,

M_1= mass in 'g' of the residue, M2= mass in 'g' of the material taken for test

2.3.3.9 Determination of acid value: For the determination of acid value, 10g of the formulation is dissolved in a neutralized mixture of alcohol and ether titrated with 0.1 M potassium hydroxide until a faint pink color appears, and the acid value is calculated using the formula.

$$\text{Acid value} = n\times 5.61/w$$

Where, n = number of ml of 0.1 N sodium hydroxide required

w = Weight in gram of the formulation

2.3.3.10 Determination of saponification value: For the determination of the saponification value, two gram of formulation is refluxed with 25 ml of 0.5 N alcoholic KOH, titrated with 0.5 N HCl using phenolphthalein indicator, and calculated using the formula.

$$\text{Saponification value} = (b-a)\times 28.5/w$$

Where,

w = weight in gram of the formulation.

2.4 Formulation and evaluation of ethosome loaded cream

2.4.1 Formulation of Ethosomes loaded cream: On the basis of physicochemical parameters of cream base an optimized base was selected for loading of resveratrol ethosome. Different doses of ethosomes containing resveratrol for loading in cream base were selected on the basis of literature survey. Optimized suspension of ethosomes equivalent to 0.05%, 0.075%, 0.1% and 0.125% was added in aqueous phase shown in **Table No.3.**

2.4.2 Evaluation of Ethosome loaded cream: The ethosome loaded creams were evaluated for physical appearance, grittiness, pH, spreadibility, viscosity, acid value, total residue content as per method reported in section 2.3.2. Creams were also evaluated for drug content, ex-vivo diffusion study, skin retention using goat skin, microbial examination and stability study.

2.4.2.1 Drug content: To determine the drug content, a 1g sample was placed in a 100ml volumetric flask and diluted with ethanol up to the mark. The flask was then shaken to dissolve the drug in the solvent. The solution was filtered through whatmann filter paper (#1), and the absorbance was measured using a UV-Visible spectrophotometer at 307 nm, against a similarly treated blank.

Table 3: Formulation of resveratrol loaded ethosomes cream formulations.

S.no	Ingredients(%w/w)	EB1	EB2	EB3	EB4
1	E3 (equivalent to resveratrol)	0.05	0.75	1.0	1.25
2	Cetyl alcohol	2.5	2.5	2.5	2.5
3	Stearic acid	9	9	9	9
4	Propylene glycol	5	5	5	5
5	Glycerine	5	5	5	5
6	Methyl paraben	0.1	0.1	0.1	0.1
7	Propyl paraben	0.05	0.05	0.05	0.05
8	Triethanolamine	2	2	2	2
9	Double Distilled Water (QS)	Upto 100	Upto 100	Upto 100	Upto 100

2.4.2.2 Ex- vivo diffusion study using goat skin: For the ex-vivo diffusion study using goat skin, a Franz diffusion cell assembly was utilized. The shaven part of the goat skin was prepared and washed with normal saline. Afterward, the excised skin was mounted on the diffusion cell assembly, positioning the stratum corneum toward donor compartment. The formulation, containing a weight equivalent to 1 mg of the drug, was placed over goat skin in the donor compartment. The receptor compartment contained of 30 ml of pH 6.8 phosphate buffer, maintained at 37°C. At suitable time intervals, one milliliter of sample was withdrawn and replaced immediately with an equal volume of fresh diffusion medium. The experiments were conducted in triplicate, and the cumulative amount of resveratrol permeated through the goat skin was plotted against time.

2.4.2.3 Skin retention study: Following the in-vitro diffusion study, the residual formulation on the epidermal layer was removed and diluted in 100 ml of ethanol. Incisions were made on the remaining skin, which was subsequently minced and combine with 100 ml of ethanol. After 24 hours, both solutions underwent spectrophotometer to determine the quantity of drug retained in skin and cream [23, 24].

2.4.2.4 Drug release kinetics and mechanism: Drug release kinetics and mechanism were analyzed by fitting cumulative percent release data to various equations: Zero order (Cumulative percentage drug release vs time), first order (log cumulative percentage drug release vs time), Higuchi (cumulative percentage drug release vs √time), and Peppas exponential equation (log cumulative percentage drug release vs log time). The release exponent (n) from the Peppas exponential equation characterizes the drug transport mechanism. A value of 'n' ≤ 0.5 signifies Fickian diffusion-controlled release. 'n'> 0.5 < 1.0 indicates anomalous transport, while 'n' = 1 or > 1 indicates case-II transport, or super case-II transport, respectively [28, 29, 30].

2.4.2.5 Microbiological Evaluation: Microbial examination of the formulated formulations followed Indian Standards methods IS 11648; 1999. Total viable counts, encompassing bacterial, yeast and mould counts were assessed using a colony counter. Bacterial counts were determined with soyabean casein digest agar, while mould and yeast estimation employed peptone agar medium as per Indian Pharmacopoeia standards. Formulations were diluted 1: 10 in sterile broth with appropriate neutralizers. 1 ml of each diluted sample was plated onto soyabean agar for bacterial growth and onto sabouraude dextrose agar for fungi, yeast, and mold. Incubation was carried out at 20-35°C for 48 hours for bacterial growth and for 5 days for fungal, yeast, and mould growth [31].

2.4.2.6 Stability study: Stability studies involved filling the prepared cream formulations into collapsible tubes and storing them at ambient temperatures (30°C and 40°C). After one month, the cream formulations were assessed for phase separation, physical appearance, and drug stability[26].

3. RESULT AND DISCUSSION

3.1 Preformulation study: Resveratrol was found to off-white powder with distinctive odor**.** The melting point of resveratrol was found to be 253°C. The melting range of resveratrol reported in literature is 253-255°C. It indicates the purity of the drug.

3.1.1 Determination of λ_{max} and preparation of calibration curve: The absorption maximum of resveratrol was found to be 307 nm in ethanol (Fig. **1a**) and pH 6.8 phosphate buffer (Fig. **1b**). The calibration curve data and plot of resveratrol in ethanol is shown in Table No **4 and** Fig **2**, respectively [15].

Fig 1. UV spectra of Resveratrol (a) Spectra in ethanol (b) Spectra in pH 6.8 Phosphate buffer

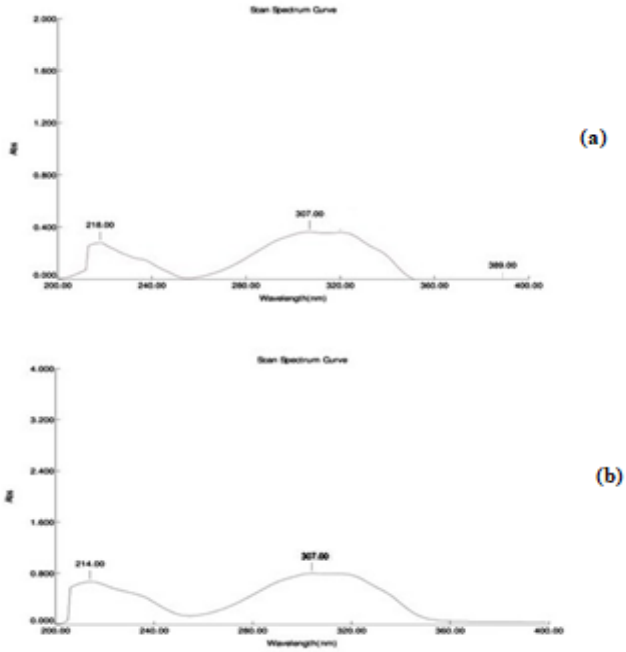

Fig 2. Calibration curve of Resveratrol in ethanol at 307 nm.

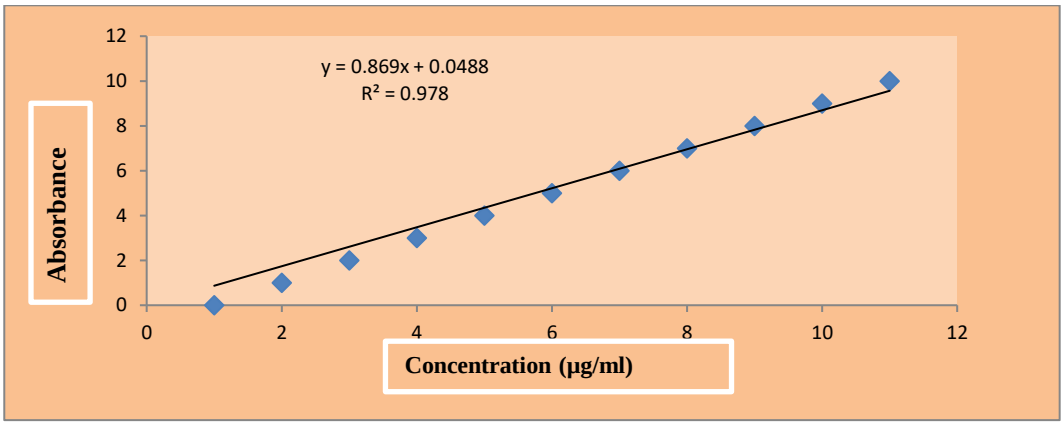

Table 4: Calibration curve data of resveratrol in ethanol at 307 nm.

Sr. No.	Concentration (µg/ml)	Absorbance ± SD
1	0.5	0.131 ± 0.007
2	1.0	0.259 ± 0.001
3	1.5	0.324 ± 0.005
4	2.0	0.398 ± 0.007
5	2.5	0.491 ± 0.008
6	3.0	0.587 ± 0.005
7	3.5	0.663 ± 0.005
8	4.0	0.749 ± 0.006
9	4.5	0.826 ± 0.005
10	5.0	0.886 ± 0.007

Mean ± SD; (n=6)

3.1.2 Determination of drug solubility: The resveratrol was found to be freely soluble in ethanol and methanol. It was practically insoluble and very slightly soluble in distilled water and pH 6.8 phosphate buffer [17].

3.1.3 Fourier transformed infrared (FTIR) studies: The FTIR spectrum of resveratrol (Fig. **3a**) shows important peak at (Phenolic -OH group stretching) 3286.4cm^{-1}, (Aromatic C-H group stretching) 3025.2cm^{-1}, (Aliphatic C=C) 1582.1cm^{-1} and (Aromatic C=C) 1134.5cm^{-1}. The detailed study of peak position for different functional groups in comparison with standard indicated the purity of the resveratrol [18].

3.1.4 Drug-excipient compatibility study: The FTIR spectrum of physical mixture of drug and excipients used in the formulation of ethosomes and cream showed (Fig. **3d**) important peak at (Phenolic -OH group stretching) 3288.4 cm^{-1}, (Aromatic C-H group stretching) 3024.6 cm^{-1}, (Aliphatic C=C) 1589.1 cm^{-1} and (Aromatic C=C) 1142.5 cm^{-1}. There is no significant variation in the peak positions of resveratrol was observed, indicating the compatibility of drugs with excipients used in the formulation of ethosomes.

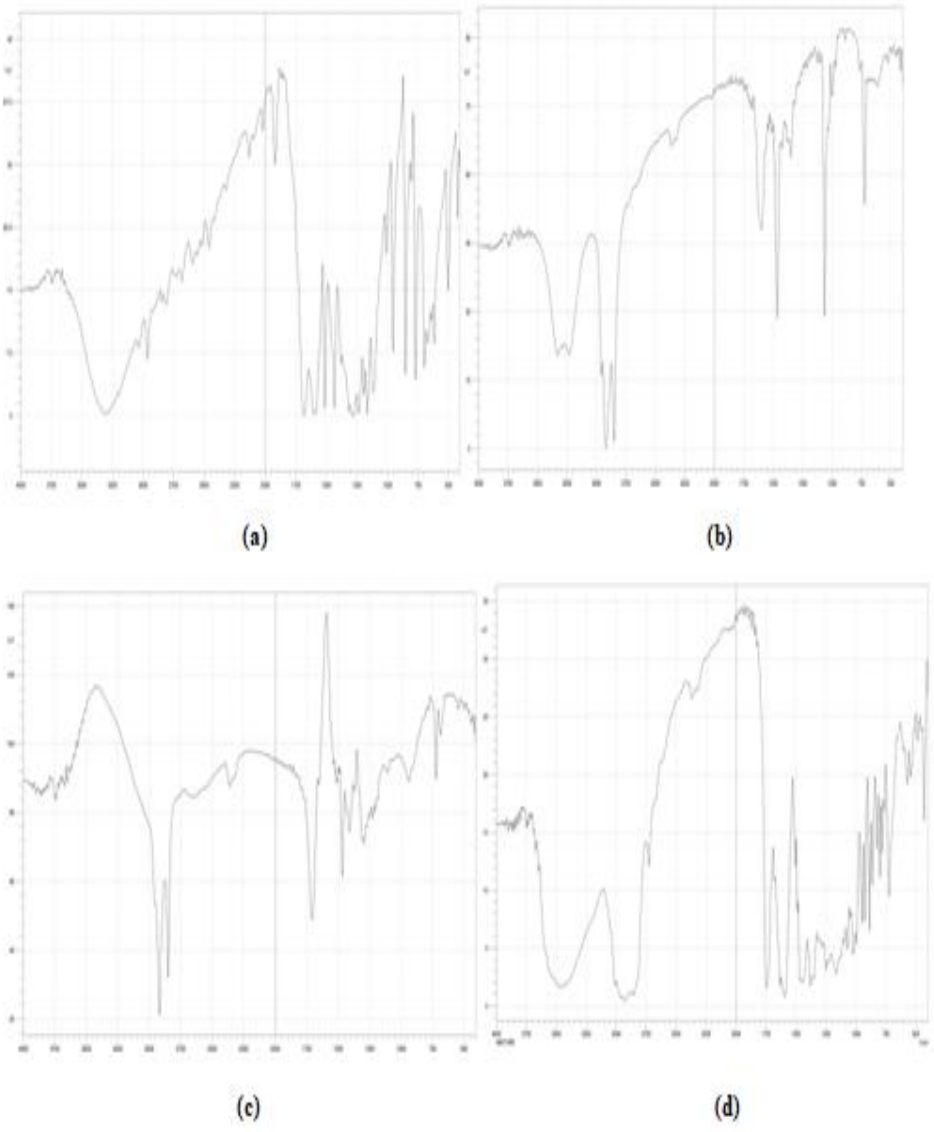

Fig 3. FTIR spectra (a) Resveratrol (b) Phosphatidylcholine (c) Cholesterol (d) Physical mixture of Resveratrol and excipients.

3.2 Formulation and evaluation of resveratrol loaded ethosomes:

Resveratrol loaded ethosomes was prepared using varying concentration of cholesterol and phosphatidylcholine by using cold approach. Formulated ethosomal formulations are observed under an optical microscope with the magnification power of 45X are shown in Fig **4**. The TEM images confirmed that the developed ethosomes were discrete and spherical in shape is shown in Fig **5**. The ethosomes of E3 and E4 were phosphatidylcholine was used in 3% concentration were more discrete and spherical in shape. In E1 and E3 no sedimentation was seen after 30 days shown in Table No.**5**. In E2 and E4 there was no sedimentation upto 25th day but, sedimentation was observed after 30th day. The sediment observed after 30th day was easily redespersible in E2 and E4. It indicated that with increased cholesterol concentration, the rate of sedimentation increased. The entrapment efficiency (%) of all the formulations was found to be satisfactory, but the maximum was for E3 shown in Table No. **6**. The percent cumulative release of the drug decreased with increased amount of phosphatidylcholine and cholesterol in ethosomes formulation. The release was controlled for the 24 hours shown in Table No.**7** and Fig **6**. On the basis of entrapment efficiency (%), TEM analysis and sedimentation in ethosomal suspension formulation E3 was selected for further investigation of particle size distribution and zeta potential. The formulation E3 was further loaded in the optimized cream base to obtain a topical cream containing ethosomes of resveratrol. The stability of dispersion can be assured on the basis of zeta potential results shown in Table No. **8, 10** and Fig. **8**. As the zeta potential increases the charged particles repel one another and they become more stable against aggregation. The stability of dispersion can be assured on the basis of polydispersity index results shown in Table No. **8**, **9** and Fig. **7**.

Fig 4. Optical microscopy of ethosomes of resveratrol.

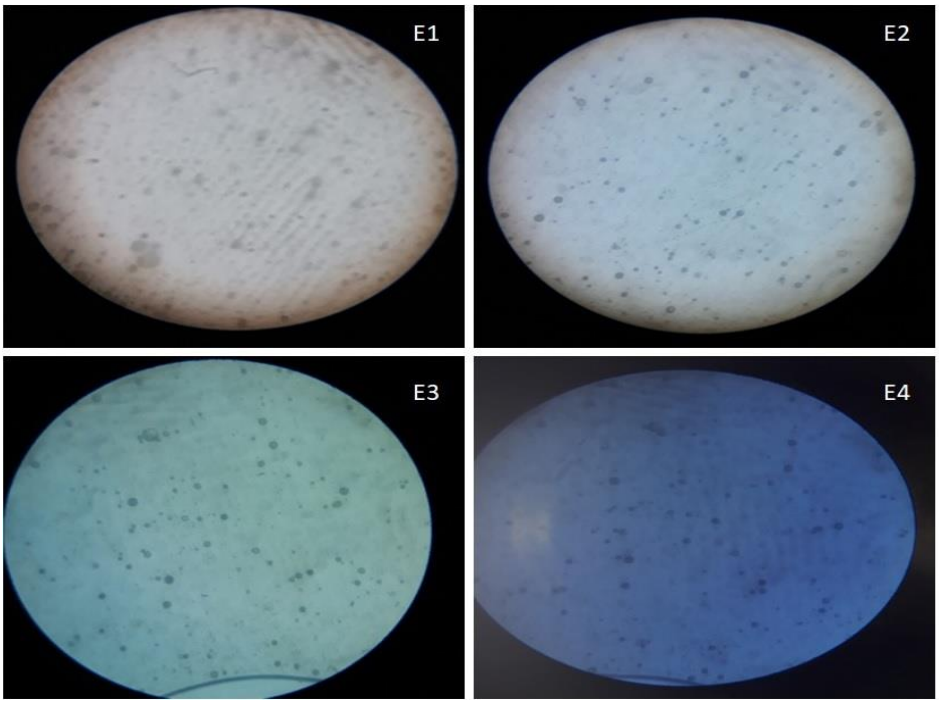

Fig 5. Transmission electron microscopy (TEM) of ethosomes of resveratrol.

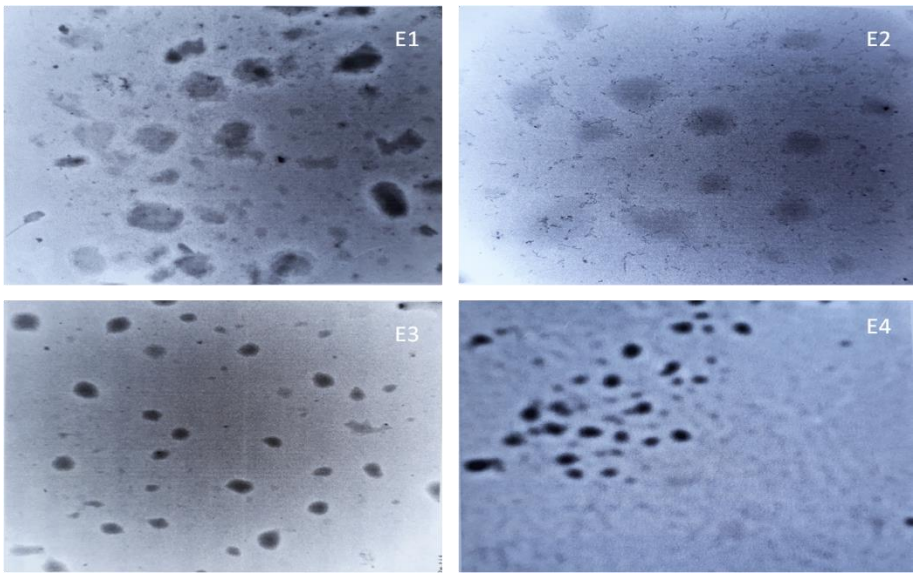

Fig 6. Cumulative percent release of resveratrol from ethosomal formulations.

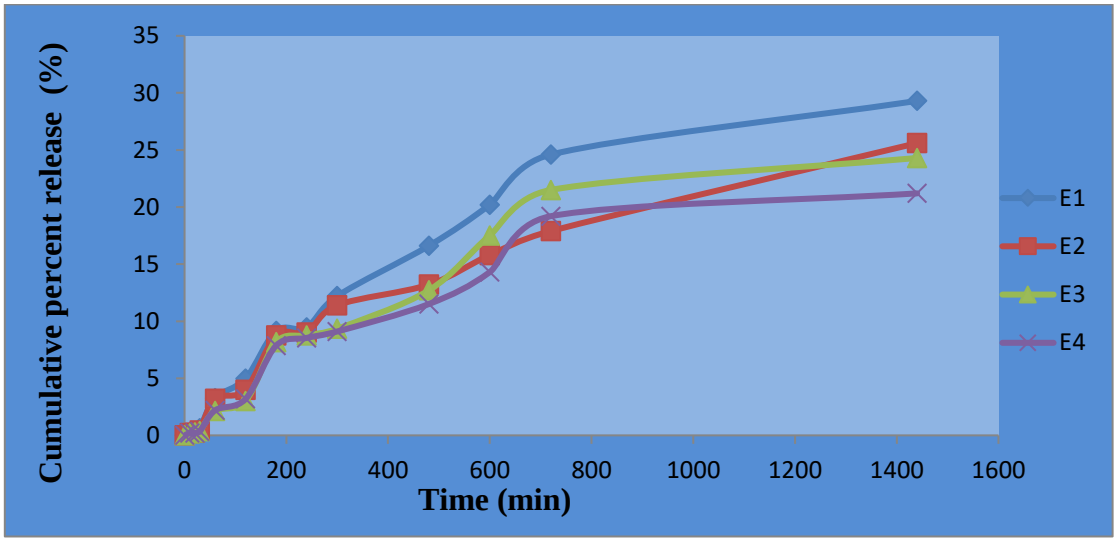

Fig 7. Results of particle size distribution measurements of the optimized ethosomal formulation stored at 2-8 ºC.

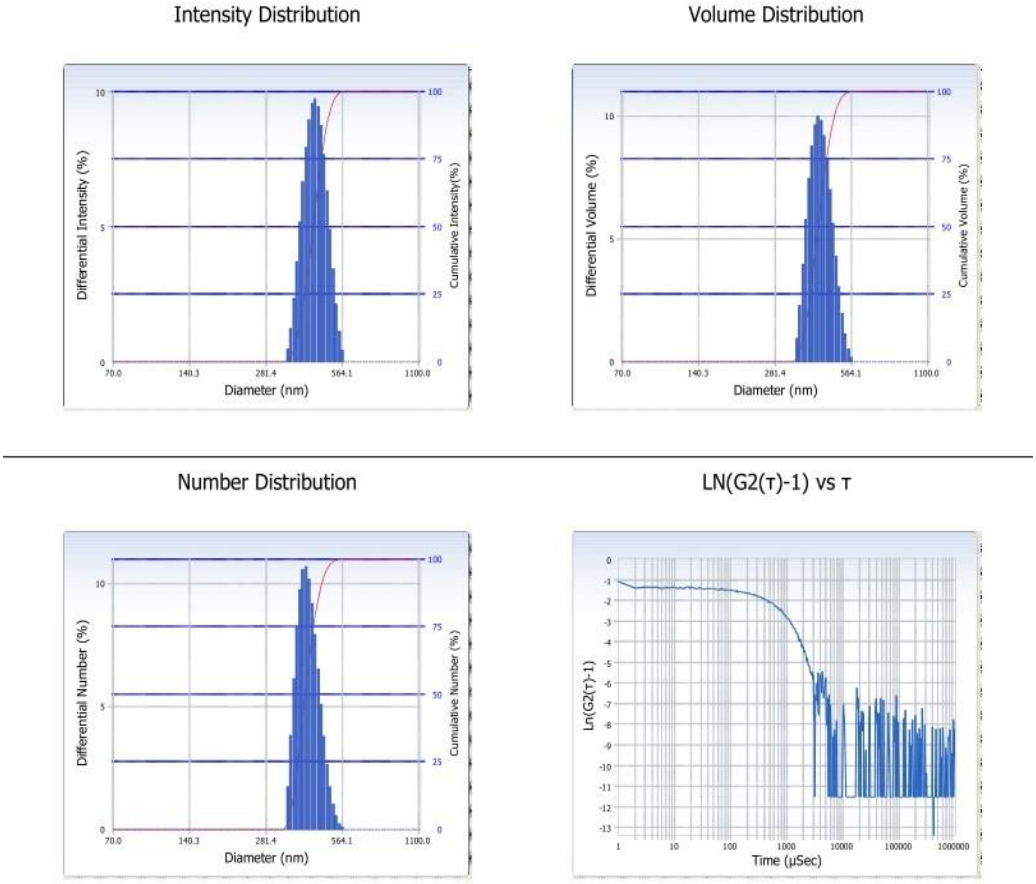

Table 5: Results of sedimentation study of ethosomal formulations.

Formulation code	Days					
	0	5	10	15	25	30
E1	-	-	-	-	-	-
E2	-	-	-	-	-	+
E3	-	-	-	-	-	-
E4	-	-	-	-	-	+

- No sedimentation, + easily redespersible

Table 6: Results of entrapment efficiency of ethosomal formulations.

Parameter	Formulations			
	E1	E2	E3	E4
Entrapment efficiency (%)	89.5 ± 0.012	96.5 ± 0.008	97.33 ± 0.015	92.8 ± 0.011

Mean ± SD (n=3)

Table 7: Diffusion study of resveratrol loaded ethosomes using dialysis membrane.

S.no	Time (min)	%CPR			
		E1	E2	E3	E4
1	10	0.23 ± 0.052	0.20 ± 0.009	0.18 ± 0.005	0.19 ± 0.021
2	20	0.30 ± 0.042	0.24 ± 0.002	0.25 ± 0.016	0.21 ± 0.052
3	30	0.62 ± 0.046	0.43 ± 0.007	0.37 ± 0.008	0.35 ± 0.086
4	60	3.26 ± 0.041	3.21 ± 0.068	2.14 ± 0.043	1.79 ± 0.095
5	120	4.98 ± 0.085	3.99 ± 0.012	3.01 ± 0.005	3.19 ± 0.032
6	180	9.15 ± 0.026	8.74 ± 0.001	8.16 ± 0.006	7.86 ± 0.001
7	240	9.45 ± 0.012	9.02 ± 0.004	8.76 ± 0.035	8.54 ± 0.033
8	300	12.2 ± 0.005	11.4 ± 0.056	9.34 ± 0.009	9.10 ± 0.041
9	360	16.6 ± 0.043	13.2 ± 0.023	12.7 ± 0.021	11.5 ± 0.009
10	480	20.2 ± 0.033	15.8 ± 0.004	17.5 ± 0.003	14.3 ± 0.002
11	600	24.6 ± 0.019	17.9 ± 0.075	21.5 ± 0.001	19.2 ± 0.007
12	720	29.3 ± 0.056	25.6 ± 0.068	24.3 ± 0.009	21.2 ± 0.004
13	1440	61.2 ± 0.014	54.4 ± 0.034	51.3 ± 0.075	46.2 ± 0.006

Mean ± SD (n=3)

Table 8: Evaluation of resveratrol loaded ethosomes for particle size distribution and zeta potential.

S.no	Parameters	Results
1	Particle size	451 ± 20 nm
2	Polydispersity index	-0.101
3	Zeta potential	-9.86

Table 9: Polydispersity index study results of the optimized formulation stored at 2-8 ºC.

Cumulants Results

Diameter	(d)	: 451.1	(nm)
Polydispersity Index	(P.I.)	: -0.101	
Diffusion Const.	(D)	: 1.091e-008	(cm²/sec)
Residual		: 4.748e-003	(O.K)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 9849	(cps)

Distribution Results (Contin)

Intensity Distribution			Volume Distribution			Number Distribution		
Peak	Diameter (nm)	Std. Dev.	Peak	Diameter (nm)	Std. Dev.	Peak	Diameter (nm)	Std. Dev.
1	440.5	45.8	1	426.6	44.0	1	413.8	41.3
2	0.0	0.0	2	0.0	0.0	2	0.0	0.0
3	0.0	0.0	3	0.0	0.0	3	0.0	0.0
4	0.0	0.0	4	0.0	0.0	4	0.0	0.0
5	0.0	0.0	5	0.0	0.0	5	0.0	0.0

Table 10: Zeta potential study results of the optimized formulation stored at 2-8 ºC

Measurement Results

Zeta Potential	: -9.85	(mV)	Doppler shift	: 5.78	(Hz)
Mobility	: -7.667e-005	(cm²/Vs)	Base Frequency	: 127.4	(Hz)
Conductivity	: 3.3495	(mS/cm)			
Zeta Potential of Cell			Diluent Properties		
Upper Surface	: -1.78	(mV)	Diluent Name	: WATER	
Lower Surface	: 4.33	(mV)	Temperature	: 24.9	(°C)
Cell Condition			Refractive Index	: 1.3328	
Cell Type	: Flow Cell		Viscosity	: 0.8898	(cP)
Avg. Electric Field	: -15.60	(V/cm)	Dielectric Constant	: 78.3	
Avg. Current	: -2.61	(mA)			

3.3 Formulation and evaluation of cream:

The results of cream base formula are shown in Table No. **11**. The consistency of formulation F1 to F3 was not good. Formulations showed flowable consistency. The consistency of formulation F4 to F9 was good. The color of formulations F4 to F9 was pearlescent white. All the formulations were having desired consistency for the creams. All the formulations were stable. The viscosity and spreadibility of the formulations increased with increase in concentration of cetyl alcohol and stearic acid. Critical chemical parameters to control in skin creams comprise pH (4 -9), acid value (within 3.5-6.5), saponification value, total fatty content (minimum 5% by mass of cream sample) and total residue content (minimum 10% by mass). The pH of formulations F4 to F9 was within the acceptable range. The human skin is naturally covered with an acidic mantle, but frequent washing and soap usage can disrupt this acidity. Therefore, to restore skin balance, creams should maintain an acidic pH range. The acid value indicates the level of free fatty acids and the potential for skin irritation caused by cream. Formulations F4 and F5 exhibited acceptable acid values, suggesting they might be gentle on the skin. Saponification values reflect the presence of free esters, which can impact formula stability and pH. Lower saponification values are preferable for stable cream formulations. Additionally, the total fatty content and residue content of all cream base formulations complied with the acceptable range outlined in the BIS 2004 guidelines for skin creams. Considering all the physicochemical parameters, F4 was selected for further loading of resveratrol containing ethosomes [26].

3.4 Formulation and evaluation of ethosomes loaded cream

The creams formulated were of pearlescent white in color. The results for the evaluation of physicochemical parameters of ethosomes loaded creams are shown in Table No. **12**. The consistency of all the formulations was good and acceptable. All the formulations were free from grittiness. The pH of the formulations was in the range of 5.84 ± 0.113 to 6.1 ± 0.121. The viscosities of the formulation were in the range of 2505.6 ± 21 to 2562.0 ± 12. The acid value, saponification value, total fatty content and total residue content were in acceptable range for the skin creams [26]. The values indicate that the topical creams might be non-irritant and stable. The drug content of the formulations was in the range of 93.1 ± 0.002 to 96.2 ± 0.005.

Table 11: Evaluation parameters for selection of cream formula.

Parameters	Formulation Code									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	Marketed
Consistency	Not Good	Not Good	Not Good	Good	Good	Good	Good	Good	Good	-
Appearance	-	-	-	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	-
Grittiness	-	-	-	-ve	-ve	-ve	-ve	-ve	-ve	-
Thermal stability	-	-	-	Stable	Stable	Stable	Stable	Stable	Stable	-
pH	-	-	-	5.9±0.153	6.5±0.153	7.4±0.153	6.1±0.153	6.6±0.153	7.5±0.153	
Spreadibility (g.cm/sec)	-	-	-	9.12±0.937	10.92±1.037	12.91±1.439	9.59±1.071	11.79±1.364	13.09±0.541	10.96±1.037
Viscosity (cps)	-	-	-	2512.1±30	2842.5±24	3182.3±31	2609.7±29	2938.2±32	3210.8±33	2834.4±39
Acid value	-	-	-	5.83±0.128	6.51±0.218	16.4±0.202	5.90±0.120	7.12±0.194	18.3±0.126	-
Saponification value	-	-	-	26.2 ±1.51	37.7 ± 1.02	47.4 ±1.42	30.3 ± 1.97	39.3 ±2.6	49.1 ± 2.49	-
Total fatty content (%)	-	-	-	7.0 ± 3.5	15.5 ±2.87	26.3 ±2.07	7.9 ± 2.16	17.2 ±1.27	29.4 ± 2.11	-
Total residue content (%)	-	-	-	16.7 ± 0.51	21.3 ± 0.28	31.1 ±0.46	17.9±0.79	22.7 ±0.45	33.7± 0.29	-

Mean ± SD (n=3)

The cumulative drug release (%) from the formulations is shown in Table No. **13** and Fig. **9**. The drug release in buffer was less than 17% from all the formulations after 24 hours. The drug content (%) of resveratrol in goat skin and cream remained in donor compartment indicates that the drug retention in skin was high in all the formulations, which is desirable for the topical formulations shown in Table No. **14.** The best possible release kinetics of the formulation shown in Table No. **15** and Fig. **10d** was found to be zero order. The 'n' value from Peppas's model indicated that the drug release was by anomalous transport [30].

The microbial count result shown in Table No. **16** for all formulations was less than 1000 cfu/g and was in acceptable range [31]**.** Stability study of formulations was done for 1 month. On the basis of comparison of data at 0 day and after 1 month it was concluded that there is no change in colour shown in Fig**. 11**, pH and viscosity of creams during storage. All the cream formulations were found thermally stable [26].

Estimation of drug from creams after 1 months indicates that there is no or minimal loss of drug during storage Table No. **17**.

Fig 8. Zeta potential measurements of the optimized ethosomal formulation stored at 2-8 ºC.

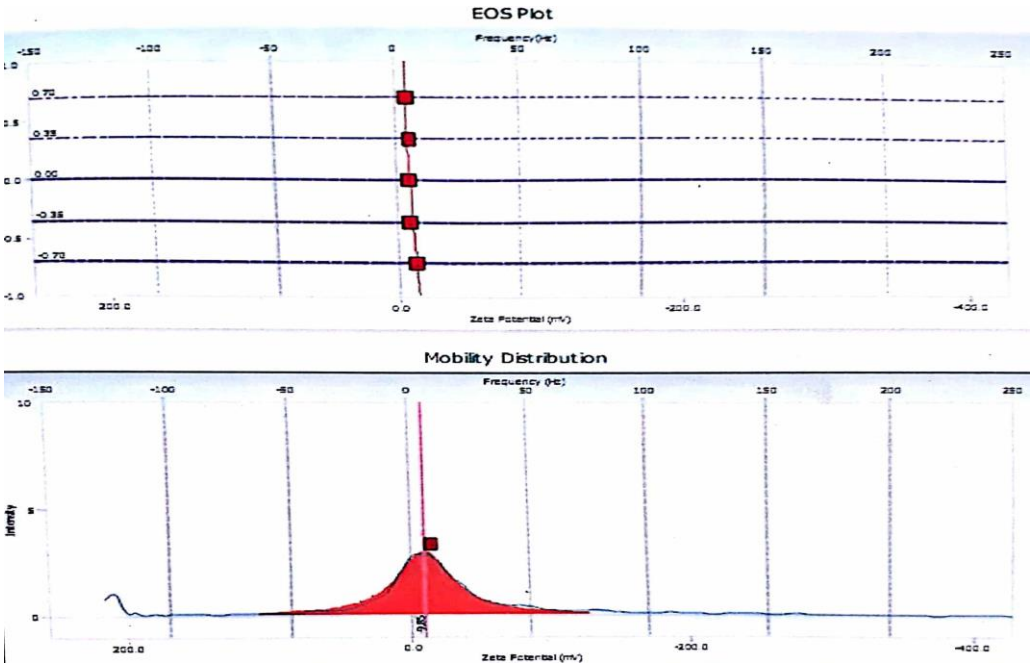

Fig 9. Cumulative percent release of resveratrol from ethosomes loaded creams.

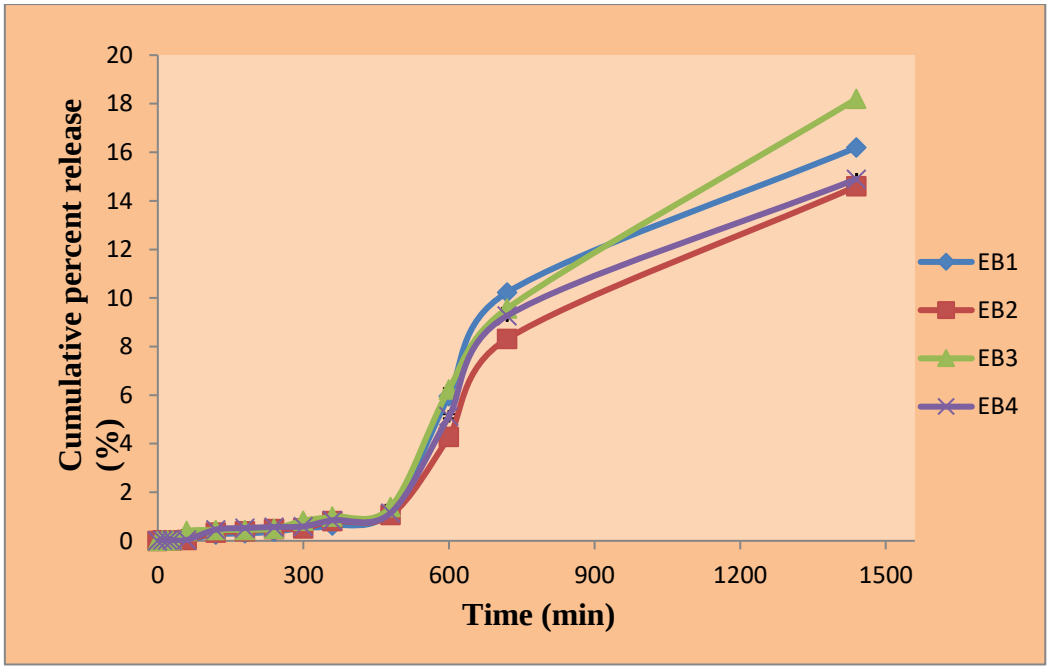

Fig 10. Model fitting and release kinetic of ethosomes loaded creams.

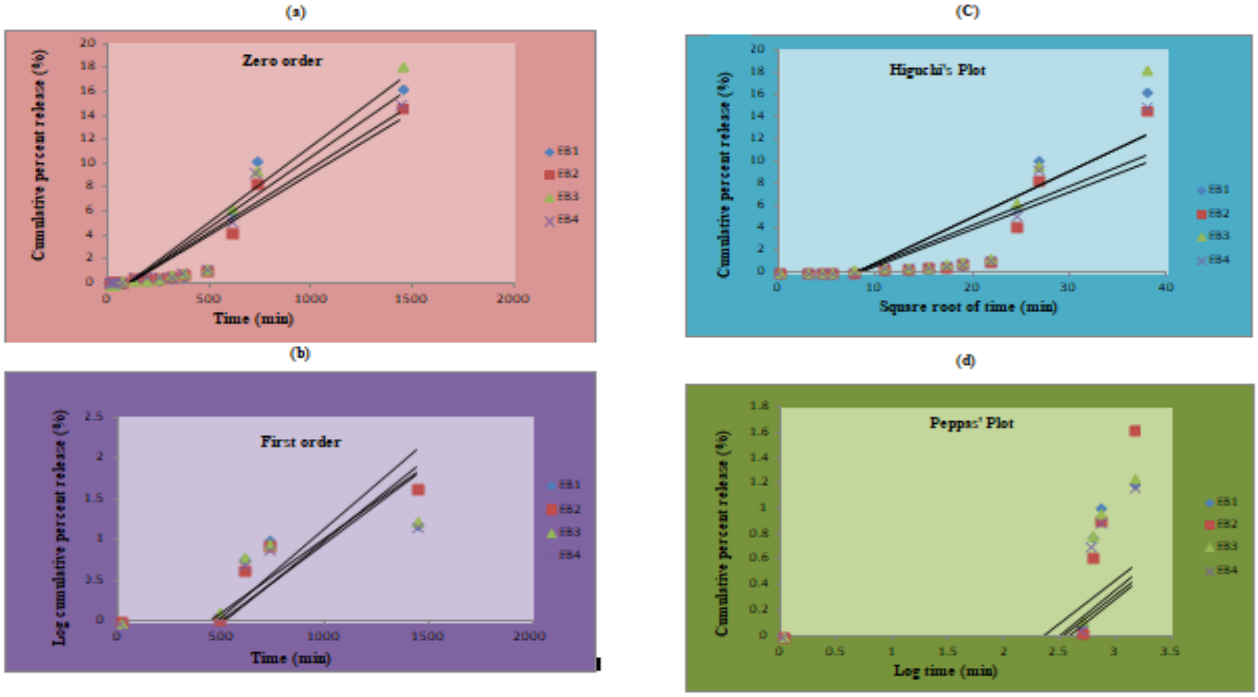

Fig 11. Ethosomes loaded cream appearance (a) at zero day , (b) after 1 month.

Table 12: Evaluation of resveratrol from ethosomes loaded creams.

S.no	Time (min)	%CPR			
		EB1	EB2	EB3	EB4
1	10	0.019± 0.005	0.024± 0.031	0.020± 0.092	0.019± 0.067
2	20	0.021± 0.016	0.0.2± 0.052	0.027± 0.032	0.024± 0.014
3	30	0.021± 0.008	0.026± 0.084	0.035± 0.076	0.030± 0.032
4	60	0.039± 0.043	0.032± 0.035	0.401± 0.011	0.034± 0.086
5	120	0.264± 0.005	0.342± 0.072	0.44± 0.035	0.462± 0.013
6	180	0.310± 0.006	0.387± 0.061	0.452± 0.076	0.527± 0.006
7	240	0.376± 0.035	0.491± 0.037	0.503± 0.012	0.570± 0.037
8	300	0.535± 0.009	0.514± 0.091	0.827± 0.005	0.592± 0.065
9	360	0.629± 0.021	0.813± 0.003	0.997± 0.034	0.846± 0.032
10	480	1.23± 0.003	1.06± 0.012	1.39± 0.073	1.15± 0.041
11	600	5.96± 0.001	4.28± 0.063	6.32± 0.091	5.13± 0.057
12	720	10.23± 0.009	8.32± 0.001	9.57± 0.056	9.27± 0.086
13	1440	16.2± 0.075	14.6± 0.035	18.2± 0.021	14.9± 0.043

Mean ± SD (n=3)

Table 13: Cumulative percent release of resveratrol from ethosomes loaded creams.

Parameters	Formulations			
	EB1	EB2	EB3	EB4
Appearance	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white
Consistency	Good	Good	Good	Good
Grittiness	-ve	-ve	-ve	-ve
Thermal stability	Stable	Stable	Stable	Stable
pH	5.84 ± 0.113	5.97 ±0.132	6.1 ± 0.121	6.0 ± 0.129
Spreadibility (g.cm/sec)	9.14 ± 0.826	9..0 ± 0.937	11.2 ± 0.912	11.6 ± 0.941
Viscosity (cps)	2510 ± 12	2505.6 ± 21	2547.4 ± 19	2562.0 ± 12
Acid value	5.83 ± 1.19	5.81 ± 1.14	5.83 ± 1.21	5.83 ± 1.09
Saponification value	26.2 ± 1.56	26.6 ± 1.02	26.1 ± 1.13	26.2 ± 1.01
Total fatty content (%)	7.0 ± 0.36	7.0 ± 0.57	7.0 ± 0.29	7.0 ± 0.87
Total residue content (%)	16.7 ± 1.29	16.7 ± 1.10	16.1 ± 1.12	16.5 ± 1.36
Drug content (%)	93.1 ± 0.122	94.7 ± 0.104	94.5 ± 0.139	96.2 ± 0.243

Mean ± SD (n=3)

Table 14: Skin retention (%) and unreleased drug (%) from ethosomes loaded cream.

Parameters	Formulations			
	EB1	EB2	EB3	EB4
Skin retention (%)	70.1 ± 0.091	68.3 ± 0.042	71.2 ± 0.023	67.3 ± 0.087
Unreleased drug from cream (%)	7.1 ± 0.028	5.3 ± 0.011	6.6 ± 0.026	9.63 ± 0.023

Mean ± SD (n=3)

Table 15: Model fitting data for release kinetics and mechanism of drug release from different formulations.

Formulations	Zero order	First order	Higuchi	Peppas's	
	R^2	R^2	R^2	R^2	N
EB1	0.889	0.674	0.6989	0.3765	0.6968
EB2	0.9	0.7481	0.6894	0.3831	0.7152
EB3	0.9097	0.6546	0.6898	0.4231	0.6778
EB4	0.8962	0.6413	0.7016	0.3917	0.687

Mean ± SD (n=3)

Table 16: Result of microbiological study of cream formulations.

Parameters	Formulations			
	EB1	EB2	EB3	EB4
Microbial count	31± 5	46 ±5	34± 1	39± 3

Mean ± SD (n=3)

Table 17: Stability data of ethosomes loaded creams at zero days and after 1 month.

Parameters	Formulations							
	At 0 day				After 1 month			
	EB1	EB2	EB3	EB4	EB1	EB2	EB3	EB4
Appearance	pearlescent white	pearlescent white	pearlescent white	Pearlescent white	pearlescent white	pearlescent white	pearlescent white	Pearlescent White
Consistency	Good	Good	Good	Good	Good	Good	Good	Good
Thermal stability	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable
pH	5.84 ± 0.113	5.97 ± 0.132	6.1 ± 0.121	6.0 ± 0.129	5.82 ± 0.123	5.99 ± 0.142	6.1 ± 0.127	6.05 ± 0.116
Viscosity (cps)	2510 ± 12	2505.6 ± 21	2547.4 ± 19	2562.0 ± 12	2562.0 ± 12	2505.6 ± 21	2547.4 ± 19	2562.0 ± 12
Drug content (%)	93.1 ± 0.002	94.7 ± 0.004	94.5 ± 0.001	96.2 ± 0.005	92.9 ± 0.005	94.0 ± 0.008	94.3 ± 0.001	95.1 ± 0.005

Mean ± SD (n=3)

4. CONCLUSION

The preformulation study provides a profile of drug and other excipients added to the formulation development, confirming their adherence to standard specifications. Consequently, these ingredients were utilized for the subsequent development of both cream base and ethosomes. The resveratrol loaded ethosomes was prepared using cold method. Different concentration of phosphatidylcholine as lipid, cholesterol as stabilizer, ethanol as penetration enhancer and solvent were selected on the basis of literature survey. Ethosomal formulations were evaluated for optical microscopy, transmission electron microscopy, entrapment efficiency, sedimentation and in vitro diffusion study using dialysis membrane. The optimized formulation on the basis of morphology, entrapment efficiency and sedimentation was further evaluated for particle size and zeta potential. On the basis of entrapment efficiency (%), TEM analysis and sedimentation in ethosomal suspension formulation E3 was selected for further investigation of particle size distribution and zeta potential. The formulation E3 was further selected for loading in the optimized cream base to obtain a topical cream containing ethosomes of resveratrol. A base cream was formulated by fusion method. Formulated creams were evaluated for organoleptic and physicochemical properties of creams. From evaluation, composition F4 (cetyl alcohol and stearic acid 2.5%w/w and 9%w/w, respectively) was selected for further loading of ethosomes.

Optimized suspension of ethosomes equivalent to 0.05%, 0.075%, 0.1% and 0.125% of resveratrol were loaded in cream base F4. The ethosomes loaded cream formulations were evaluated for physical appearance, grittiness, thermal stability, pH, spreadibility and viscosity. The formulations were further evaluated for acid value, saponification value, total fatty content and total residue.

The creams were also evaluated for drug content, Ex-vivo diffusion study using goat skin and skin retention study. The release study reveals that release was controlled for the 24 hours. The drug release in buffer was less than 17% from all the formulations after 24 hours. The results of drug content (%) of resveratrol loaded ethosome remained in donor compartment indicates that the drug retention in skin was high in all the formulations, which is desirable for the topical formulations.

The best possible release kinetics of the formulation was found to be zero order. The 'n' value from Peppas's model indicated that the drug release was by anomalous transport. . Ethosomal cream formulations were evaluated for thermal stability, physical appearance, viscosity and drug stability after 1 month. Stability study indicates that all the cream formulations were stable after a time period of one month.

The present study conclusively supported that ethosomal cream containing resveratrol to be an advantageous topical drug delivery system in treatment of contact dermatitis. However detail in- vivo study should be conducted in future to justify the in- vitro study.

CONFLICTS OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

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