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FORMULATION AND EVALUATION OF PHYTOCONSTITUENTS EMULGEL FOR THE TREATMENT OF VARICOSE VEINS

Santosh V. Gandhi*, Nikita M. Nilgar and Mangesh R. Bhalekar

AISSMS College of Pharmacy, Savitribai Phule Pune University, Pune, India-411001.

***Corresponding Author: Dr. Santosh V. Gandhi**

AISSMS College of Pharmacy, Savitribai Phule Pune University, Pune, India-411001.

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ABSTRACT

The aim of the present research work was to investigate the potential of emulgel in enhancing the topical delivery of phytocontituents rutin and quercetin. Emulgel formulations of phytoconstituents were prepared using gelling agent Carbopol 934. The influence of the gelling agent, the concentration of the oil phase and emulsifying agent on the drug diffusion from the prepared emulgel was investigated using a 3^2 factorial design. The factors chosen were the concentration of glyceryl caprylate (GC) and the ratio of surfactants as Span 80: tween 80. The prepared formulations were evaluated for viscosity, spreadability, drug diffusion, globule diameter, drug content and skin irritancy test. The optimized batch showed acceptable globule diameter (218.6 nm), spreadability (41.9 g), viscosity (7850 cps), drug content for rutin and quercetin (98.8% and 101.8%) and pH (6.7). The *in-vitro* drug diffusion and *ex-vivo* permeation were found to be higher for optimized formulation as compared to the saturated solutions of pure drugs. The result of studied emulgel reveled that the invitro flux value of optimized batchwas found 0.2316 mg/hr/cm^2 (rutin) and 0.2457mg/hr/cm^2 (quercetin) respectively. It was observed from ex-vivo permeation studies that the flux for optimized emulgel was found to be 0.5367 and 0.5297 mg/hr/cm^2 respectively as compared to the saturated solutions of rutin (0.1035mg/hr/cm^2) and quercetin (0.1264mg/hr/cm^2). While result of skin irritation test shows no edema and erythema on the skin of the rabbits. In general conclusion, it was suggested that the emulgel formulation succeed the drug release for sustained drug delivery in a controlled manner in comparison.

KEYWORDS: Rutin, Quercetin, Emulgel, Varicose veins, Exvivo permeation.

INTRODUCTION

Varicose Veins is a disease of veins leadingtothe backward flow and turbulence in the circulation of the blood.[1] The veins get perverted, become enlarged due to a condition called edema. It involves a genetic predisposition, incompetent valves, weakened vascular walls, and increased intravenous pressure. A heavy, achy feeling, itching or burning and worsening with prolonged standing are all symptoms of varicose veins.[2] It is thought to be due to reasons like menopause, obesity, standing for long period of time, pregnancy etc.[3] Prominent treatment strategies are external laser treatment, injection sclerotherapy, endovenous interventions and surgery.[4] Choice of therapy is affected by symptoms, patient preference, cost, potential for iatrogenic complications and available medical resources. Different traditional and alternative therapy for treating varicose veins, we have identified phytoconstituents like rutin and quercetin which are reported to have good effect on the veins helping them in better functioning. Rutin through its free radical activity is able protect these walls and it inhibit the PAF (platelet activating factor) and thromboxane A2 thus diminishing capillary permeability.[5,6] Quercetin dramatically stabilize small blood vessels relating to the veins, helping reduce fluid retention and specifically boost integrity of vessels.[7] Presently these phytoconstituents are administered orally in suppliments form with very high doses and which show poor bioavailability.

Therefore present study attempts to formulate these phytoconstituents as topical novel emulgel form which is patient friendly using principles of formulation design and design of experiment strategy to get a formulation with desired performance attributes.

MATERIALS AND METHOD

Materials

Rutin was acquired from Loba Chemie Pvt. Ltd., (Mumbai, India) and Quercetin was obtained from Green Heaven India Pvt. Ltd., (Nagpur, India). All other ingredients used in the formulation of cream were purchased from local market and were of extra pure grade.

Development and formulation of emulgel

Drug Excipient Compatibility[8]

Compatibility of rutin and quercetin with formulation excipients such as span 80 and tween 80 was determined by keeping them together in hermetically sealed glass vials at 40^0C for two weeks. Subsequently FTIR (Jasco FTIR4100) analysis was performed on individual components and drug excipient mixtures between 400cm^{-1} to 4000$cm^{-1}$and when paired with actives to check for any changes in the functional groups.

Determination of saturation solubility of phytoconstituents[9]

The solubility of rutin and quercetin in glyceryl caprylate, mineral oil and water was determined. Excess of rutin and quercetin were added separately to 5 mL each of glyceryl caprylate, mineral oil and water. All the six dispersions were kept for shaking in orbital shaker (Model: CIS-24 BL) for 48 h. A 1ml clear supernatant was taken and diluted suitably with ethanol and samples were analysed by UV spectrophotometer at the wavelength 257 nm and 372 nm respectively.

Selection of phytoconstituents emulgel formula

General emulgel formula was selected from literature[10] as presented in Table 1.

Table 1: Formula for emulgel.

Sr.no	Ingredients	Quantities(%)
1.	Rutin	1.0
2.	Quercetin	1.0
3.	Propylene glycol	5.0
4.	Carbapol 934	3.0
5.	Span 80:Tween 80	2.0-6.0
6.	Propyl paraben	0.3
7.	Triethanolamine	q.s.
8.	Ethanol	10.0
9.	Water	q.s.
10.	Glyceryl caprylate (GC)	20.0-30.0

Design of experiment

Experimental Factorial design (2 factors, 3 levels) was chosen to derive formula which provided optimum globule diameter, spreadability and viscosity. The factor combinations, levels and responses are shown in the table below (Table 2).

Table 2: Factors and responses chosen for the design.

Variables		Levels		
A	Glyceryl caprylate	+1	0	-1
		20%	25%	30%
B	Span80:Tween80 (3:7)	2%	4%	6%
Responses		**Goals**	**Acceptance range**	
X	Globule diameter	Optimum	200-400nm	
Y	Spreadability	Optimum	<70 Cp	
Z	Viscosity	Maximum	91-101%	

Preparation of Emulgel[11]

The RHLB value of Glyceryl caprylate was found to be 11 and accordingly the surfactant mix (Smix) chosen was Span 80: Tween 80 (3:7).

Preparation of emulsion phases

The oily phase of emulsion was prepared by dissolving span-80 in glyceryl caprylate with required quantity of quercetin in ethanol. Aqueous phase was prepared by dissolving rutin and tween-80 in purified water. Propyl paraben was dissolved inpropylene glycol and mixed with aqueous phase.

Preparation of gel

Accurately weighed quantity of carbopol-934 was taken in a previously dried beaker and 10 ml of distilled water was added to it. It was mixed well using mechanical shaker with constant stirring. More distilled water was added to it to maintain the consistency of the gel. The pH of the formulation was adjusted to 6.0 to 7.0 using triethanolamine.

Formulation of emulgel

Both the oily and aqueous phases were separately heated to 40^0C to 50^0C, than mixed with the continuous stirring and allowed to cool to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain a phytoconstituents emulgel formulation.

Evaluation of Phytoconstituents Emulgel[12]

(1) Determination of pH: pH of 1% dispersion of cream and emulgel in Glycerine:water (1:1) was measured using pH meter [Deluxe101].

(2) Determination of globule diameter

The globule diameter of 1% dispersion of cream and emulgel in glycerine:water(1:1) was measured by using Malvern Zetasizer ZS 90 UK.

(3) Spreadability

Hardness of formulation was determined using a texture analyzer (Brookfield CT-3) emulgel (20 gm) was filled in conical probe (up to plane surface of top) and hardness was measured. This apparatus shows hardness into the comparison of spreadablity and adhesive force.

(4) Viscosity

The viscosity of the formulation was determined using viscometer (Brookfield digital viscometer RVDV Pro) equipped with ULE adapter. The spindle (S-06) was rotated at 50 rpm. Samples of the emulgel were allowed to settle over 30 min at the temperature (25 ± 10^0C) before the measurements were taken. Viscosity was reported in (cP).

(iii) Analysis of drug content by first derivative spectroscopy[13]

Accurately weighed 1g of formulated emulgel sample was transferred to 10 ml volumetric flask and volume was adjusted using ethanol. The resulting solution was filtered using 0.45 µm filter and suitably dilutedwith ethanol. This solution was then analyzed at the wavelength 257 and 372 nm using UV Spectrophotometer (Jasco V730) and the above spectra was further converted to first derivative spectroscopy and the absorbance of rutin was recorded at zero crossing point of quercetin (372 nm) and the absorbance of quercetin was recorded at the zero crossing point of rutin (257 nm) to determine the content of rutin and quercetin in the formulation respectively.

(iv) *In-vitro* drug diffusion[14]

The release of drug from the emulgel was determined using Franz diffusion cell apparatus for 6h.The receptor medium was hydroalcoholic solution containing phosphate buffer and ethanol in the ratio (7:3) at pH 7.4, maintained at 37^0C. The membrane filter used was (cellulose acetate) membrane pore size 0.45µ and soaked in hydroalcoholic solution pH 7.4 for 1h. The membrane was mounted between the donor and receptor compartment. The emulgel (300 mg) was placed on receptor compartment and both the compartments were clamped together. The hydroalcoholic solution having pH 7.4 in the receptor compartment (8 ml) was stirred using magnetic stirrer 60 rpm. Aliquots of 1 ml were withdrawn after each interval and the same amount of solution was replaced with the fresh hydroalcoholic solution pH 7.4. Collected samples were filtered through 0.45µm filter. Rutin and quercetin were quantified by using HPLC at 255nm.[14] Saturated solution of pure rutin and quercetin in pH 7.4 phosphate buffer were used as standards since topical marketed formulation of rutin and quercetin was not available. The amount drug release was calculated.

(v) *Ex-vivo* drug permeation studies

For *exvivo* permeation studies abdominal shaved skin (3.14 cm2) of excised male Sprague-Dawley rat weighing 200-250 g was used and the procedure was followed as above for *invitro* diffusion. The amount drug release was calculated.

HPLC analysis of sample[15]

Rutin and quercetin concentrations in the diluted solutions were determined by HPLC using (HPLC system used was JASCO system equipped with model PU 2082 Plus pump, Rheodyne sample injection port (20 µl), JASCO UV 2075 Plus detector and Borwin chromatography software (version 1.5) with HiQSil C18 (250mm*4.6 mm, 5µm) column. The mobile phase was a mixture of acetonitrile : water (60:40), water pH adjusted to 3 by orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and pumped from the filter reservoir at a flow rate of 1 ml/min which yielded a column back pressure of 110-120 bars. The run time was set at 10 min and the volume of injection was 20 µl. The column was equilibrated for at least 30 min with the mobile phase running through the system before injecting the drug solution. Then the samples withdrawn from franz diffusion cell were diluted with the mobile phase filtered through sample filter and injected into the column. The eluent was monitored by isocratic elution at 255 nm.

(v) Skin irritancy test[16]

The animal study protocol was reviewed and approved by the Institutional Animal Ethical Committee, AISSMS College of Pharmacy (Regd no. 257/P0/ReBi/S/2000/CPCSEA, Dated-13/01/18), Savitribai Phule Pune University, Pune. The formulation containing the lowest effective strength was tested on wistar rats. As follows: each rat was kept in different cage food was supplied during the test period. 24 hours prior to test the hair from the spine region was shaved. The test site was cleaned with surgical spirit then 1gm emulgel was applied to test area. The test site was observed for erythema and odema after 24hrs after application.

RESULTS AND DISCUSSION

Drug Excipient Compatibility

The FTIR spectrum of Rutin and Quercetin respectively was recorded and compared with that of the standards in the literature. The major peaks of rutin and quercetin could be seen as mentioned in the table-3 below while there was absence of any new peak other than that for rutin and quercetin peaks. From the spectra it can be concluded that there is absence of any Well-defined

interaction between the drugs and the excipients. Hence, drug – excipients compatibility was established.

Table 3: Interpretation of IR spectrum rutin and quercetin.

Interpretation of rutin		Interpretation of quercetin	
Wave number (cm^{-1})	**Functional group**	**Wave number (cm^{-1})**	**Functional group**
3439, 3321	-OH Stretching	3420, 3369	-OH Stretching
2924,2861	-CH_2 Stretching	2986	-CH_2 Stretching
2714	-CH bonding	2872	-CH stretching
1402	-C=O Vibration	1457	-(C=O) Stretch
1383	-C-OH Vibration	1362	-C-OH Vibration

Determination of saturation solubility of phytoconstituents

Highest solubility of rutin was seen in water (30mg/ml) and that of quercetin was seen in glyceryl caprylate (40mg/ml). Hence, glyceryl caprylate was selected as internal phase. The solubilities are as follows in Fig. 1.

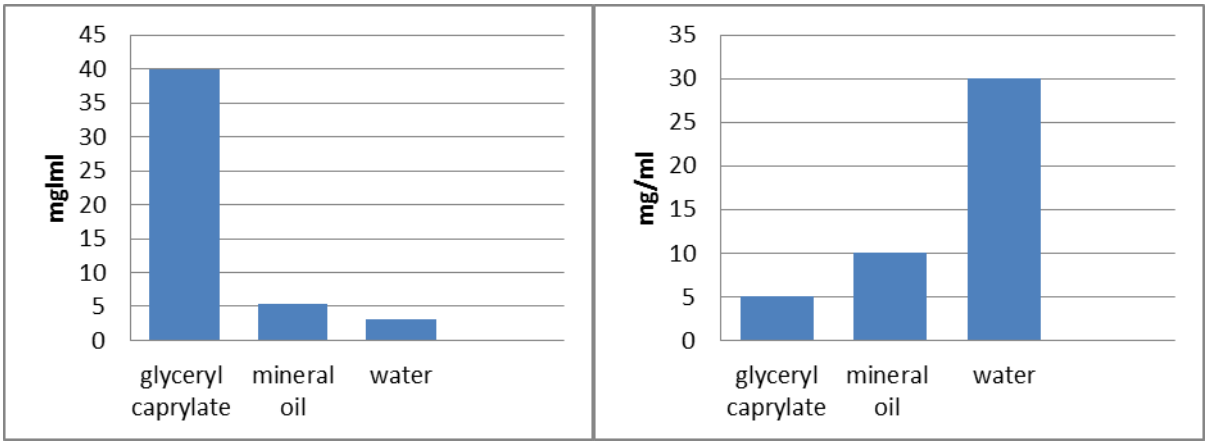

Fig. 1: Solubility of rutin and quercetin in differents solvents.

Design of experiment

Optimization of the formula (Table 1) was done using 2 factor 3 level factorial design using Design Expert 11.0 trial version. The responses such as globule diameter, spreadability and viscosity were chosen for optimization as globule diameter with affects the surface area which indirectly affects viscosity, spreadability increases the ease on application and viscosity governs the drug release/ diffusion from the formulation. These factors depend mainly on quantity of oil phase and the alkalizing agent hence were selected for optimization using three levels give good idea about interaction between factors if any.

Evaluation of phytoconstituent cream (Table 4)

Gelling agent is the agent which helps in formation of matrix necessary for entrapment of the drug. The concentration of gelling agent is important for aesthetic properties of the gel. The concentration decides the viscosity of the formulation which in turn affects the permeation of the drug across the skin.

Table 4: Evaluation of trial runs of factorial design.

Sr. No	Formulation code (A %, B %)	Globule diameter (nm)	Spreadability (Hardness) G	Viscosity (Cps)	pH
1	F1 (20.0, 2.0)	235.3	37.4	7585	6.3
2	F2 (25.0, 2.0)	234.6 142.2	29.87	9860	6.5
3	F3 (30.0, 2.0)	142.2	28.95	10100	6.4
4	F4 (20.0, 3.0)	236.8	44.88	8189	6.7
	F5 (25.0, 3.0)	309.6	42.6	7990	6.8
6	F6 (30.0, 3.0)	218.6	41.98	7850	6.7
7	F7 (20.0, 4.0)	215.3	38.9	10800	6.7
8	F8 (25.0, 4.0)	272.4	41.6	11250	6.9
9	F9 (30.0, 4.0)	236.8	56.4	8990	6.5

1. **Determination of pH:** The pH of all the batches of emulgel was determined to establish the non-irritancy of the cream *in vitro*. Normally, the pH of skin is in the range of 6.0-7.0 and all the values in this range can be said to be appropriate for emulgel formulation, thus here it was concluded that the cream is non-irritant to the skin *in vitro*.
2. **Determination of Spreadability:** The spreadability of emulgel is very important aesthetic property. It establishes the patient friendliness of the cream. Brookfield texture analyser measures hardness of the formulation which is indicative of cohesiveness of formulation, higher hardness means inability to spread.
3. **Determination of viscosity:** The viscosity is defined as the resistance to flow of the formulation. It is determined by subjecting the formulation to shear. As semisolid formulations have to remain in contact with the skin for a longer period of time, the viscosity should be high. But, if the viscosity is very high, the formulation becomes rigid and the spreadability decreases. So, it is necessary to choose optimum amount of variables to get good viscosity. The viscosity of the emulgel was seen to be affected by amount of gelling agent (Carbapol 934). As the amount increases, the viscosity of emulgel increases.
4. **Determination of globule diameter:** The globule diameter is an important evaluation parameter as it affects various performance parameters such as viscosity, spreadability and drug permeation. Smaller the globule diameter, improves absorption but very small diameters increase the viscosity and affect spreadability adversely. Hence, optimum globule diameter is necessary for formulation. The globule diameter decreased with amount of surfactant and increased with amount of carbapol 934.

Having evaluated all the parameters formulation F5 was found satisfactory.

The model terms for the emulgel globule diameter (Fig. 2) was found to be significant with high value of R^2 0.9584 which indicates the adequate to a quadratic model. Values of probability F was less than 0.5 indicated that the model terms were significant. The predicted R-squared of 0.6603 is in reasonable agreement with the adjusted R-square 0.8891; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.1 it was found that the globule diameter increases with increase in theconcentration of glycerylcaprylate and as the concentration of surfactant that is span 80: tween80 increases the spreadability increases due to increase in globule diameter.

Globule diameter = +101.54667A + 2.25000B + 2.86500AB – 2.32000A^2+ 8.06250B^2……(1)

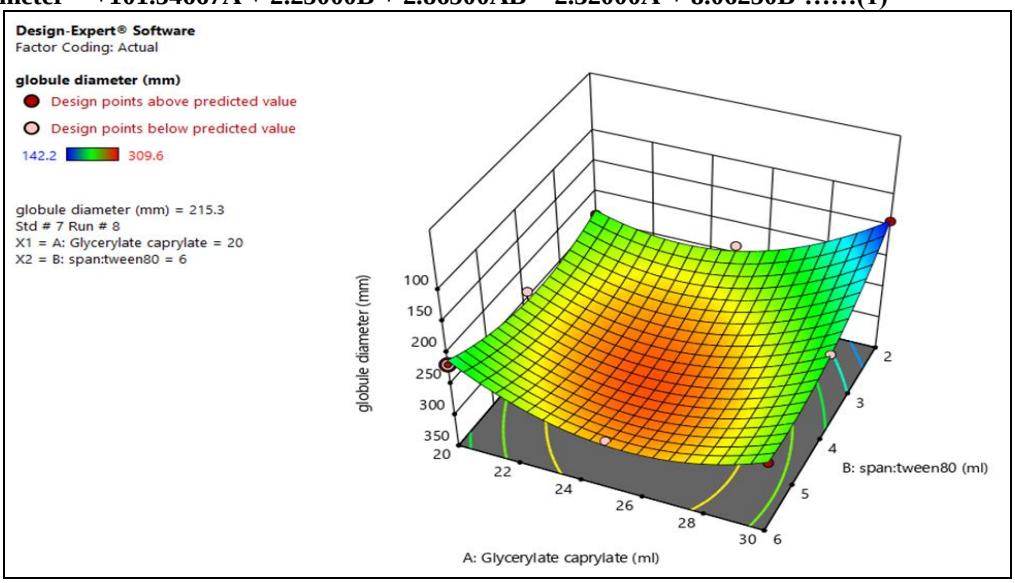

Fig. 2: Response surface depicting effect of glyceryl caprylate and Span80:tween80 on globule diameter.

The model terms for the emulgel spreadability (Fig. 3) was found to be significant with high value of R^2 0.8374 which indicates the adequate to a quadratic model. Values of probility F was less than 0.5 indicated that the model terms were significant. The predicated R-squared of 0.5798 is in reasonable agreement with the adjusted R-square 0.7398; i.e. the difference is more than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.2 it was found that the speadability increases with increase in the concentration of glyceryl caprylate as glyceryl caprylate makes the emulgel more spreadable and less viscous and as the concentration of Span80: tween80 increases the spreadability reduced as the globule diameter increases which leads to less surface area and ultimately decreases the spreadability.

Spreadability = +40.89 + 1.03A + 6.78B + 6.49AB + 3.39A2 – 4.30B2……(2)

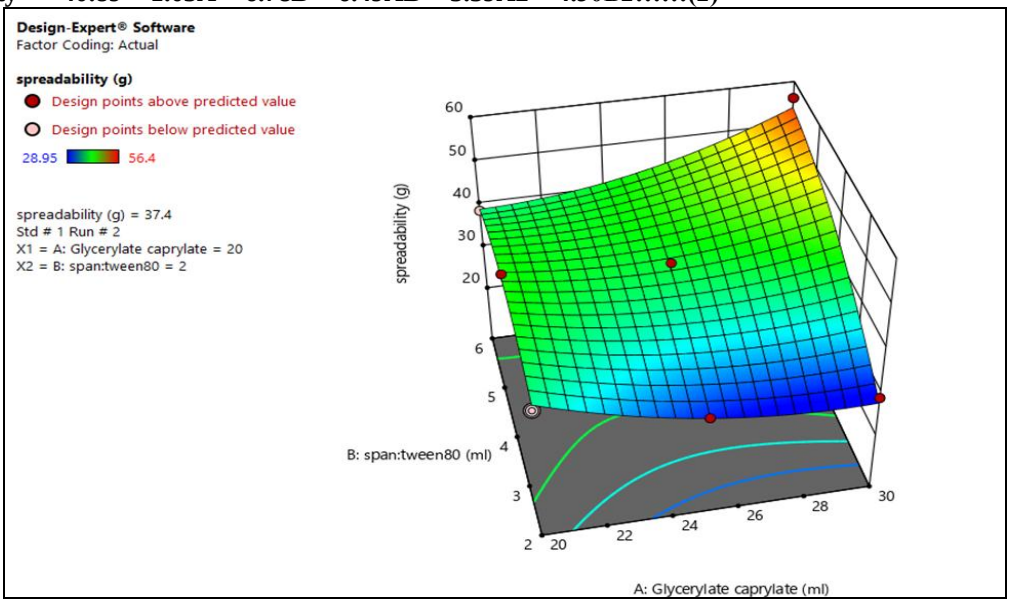

Fig. 3: Response surface depicting the effect of glycerylcaprylate and Span80:tween80 on spreadability.

The model terms for the emulgel viscosity (Fig. 4) was found to be significant with high value of R^2 0.9429 which indicates the adequate to a quadratic model. A value of probability F was less than 0.5 indicated that the model terms were significant. The predicted R-squared of 0.5127 is in reasonable agreement with the adjusted R-square 0.8478; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. From eq.3 it was found that the viscosity increases with decrease in the concentration of TEA due to decrease in the globule diameter and as the concentrationof glyceryl caprylate decreases the viscosity increases due to less concentration of oil phase.

Viscosity = +8530.33 + 61.00A + 582.50B -1081.25AB – 781.00A^2 + 1754.50B^2 ….(3)

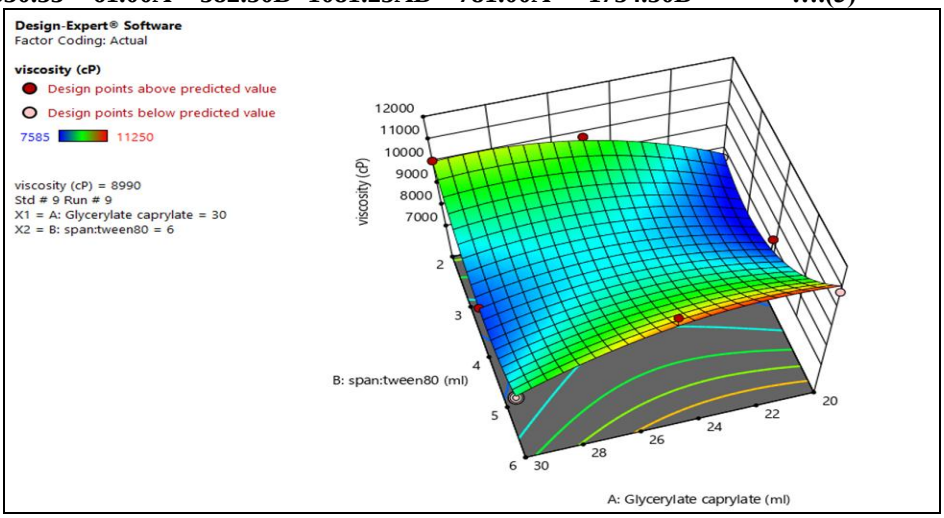

Fig. 4: Response surface depicting effect of glycerylcaprylate and Span80:tween80 on viscosity.

Drug content by first derivative spectroscopy

Drug content was calculated for all the batches (Table-5). The solutions were analyzed at 257 and 372 nm using UV Spectrophotometer and the spectra were further converted to the first derivative order to determine the absorbance of rutin at the zero crossing point of quercetin (372 nm) and quercetin at the zero crossing point of rutin (257nm) respectively.

Table 5: % Drug content of emulgel formulations.

Sr. No	Formulation code	Drug content (%)	
		Rutin	Quercetin
1	F1	46.24	14.19
2	F2	68.72	66.96
3	F3	7.76	48.093
4	F4	21.73	39.71
5	F5	30.73	35.95
6	F6	98.87	101.84
7	F7	7.5432	47.86
8	F8	31.92	36.68
9	F9	30.73	35.95

Fig. 6 demonstrate the data from *invitro* drug diffusion studies, it was observed that formulation showed better flux (0.2316 and 0.2457 mg/hr/cm^2) for rutin and quercetin respectively due to presence of excipients like glyceryl caprylate with better penetration and absorption and optimum concentration of surfactant.

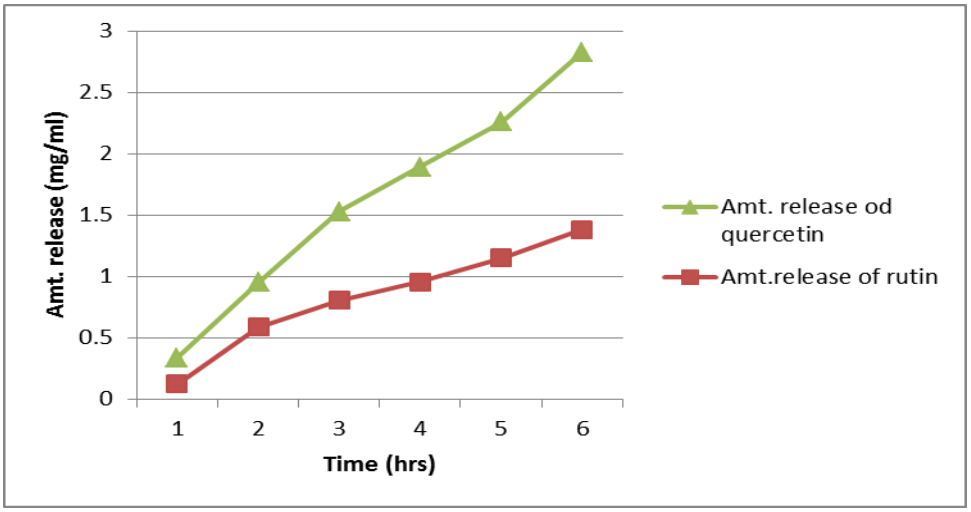

Fig. 6: Amount in vitro drug release from optimization batches.

It was observed from *ex-vivo* permeation studies that flux of drugs from optimized emulgel (rutin and quercetin) was found to be 0.5367 and 0.5297 mg/hr/cm^2 respectively as compared to the saturated solutions of pure rutin and quercetin (0.1035 and 0.1264 mg/hr/cm^2) respectively as presented in Fig. 7.

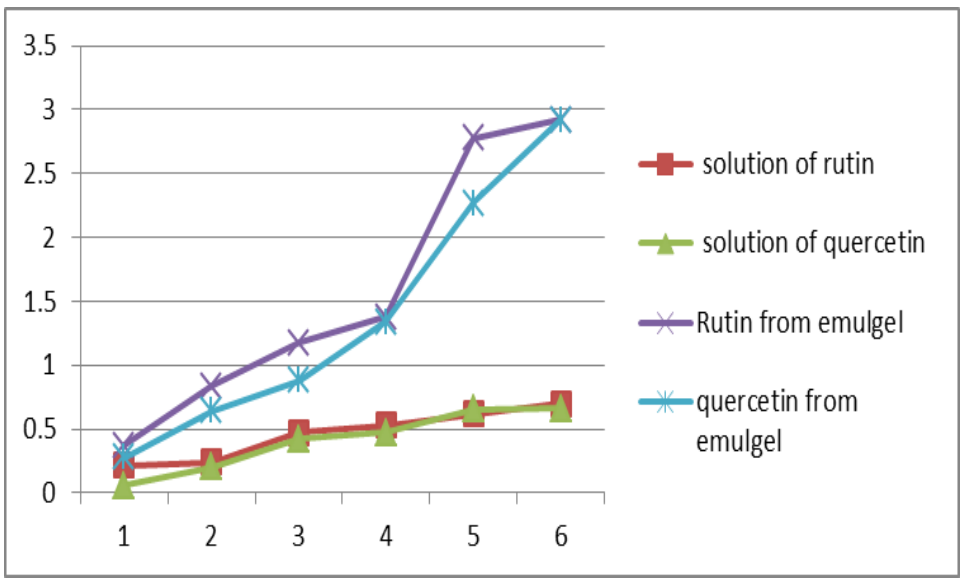

Fig. 7: Amount Ex-vivo drug release from optimized batch.

CONCLUSION

Topical emulgel formulation of rutin and quercetin has been attempted for venous diseases and varicose veins treatment which is patient compliant approach and economical. The formulation has been optimized by DOE methodology and provide higher penetration to site of action. Thus, it can be better alternative to oral administration of rutin and quercetin. This study proves the ability of phytoconstituents with potential application to treat varicose veins with consequent health benefits.

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REFERENCES

1. Varicose veins | University of Maryland Medical Center; file:///H:/varicose%20veins/Varicose%20veins%20_%20University%20of%20Maryland%20Medical%20Center.pdf
2. Varicose veins: National Heart, Lung and Blood Institute; https://www.nhlbi.nih.gov/health-topics/varicose-veins
3. Healthline: Varicose veins
4. https://www.healthline.com/health/varicose-veins
5. Bootun R, Onida S, Lane RA, Davies AH. Varicose veins and their management. Surgery (Oxford), 2016; 34(4): 165-171.
6. Brake M, Lim CS, Shepherd AC, Shalhoub J, Davies AH. Pathogenesis and etiology of recurrent varicose veins. Journal of Vascular Surgery, 2013; 57(3): 860-863.
7. Arct J, Pytkowska K. Flavonoids as components of biologically active cosmeceuticals. Clin Dermatol, 2008; 26(4): 347-57.
8. Byron J. Richards, Board Certified Clinical Nutritionist; Quercetin Helps Veins Function Better; March 24, 2012 (https://www.wellnessresources.com/news/quercetin-helps-veins-function-better)
9. Chaudhari SP, Bangar JV, Akuskar GK and Ratnaparkhi MP. Development and validation of UV spectrophotometric method for simultaneous estimation of rutin and quercetin in niosome formulation. Der Pharmacia Lettre, 2014; 6(3): 271-276.
10. Singhvi G, Sonavane S, Gupta A, Gampa G and Goyal M. Design of a Modified Kinetic Solubility Determination Method at Laboratory Level for Early Drug Discovery. Asian Journal of Chemistry, 2013; 25(1): 237-239.
11. Ranga PM, Sellakumar V, Natarajan R and Kumar MK. Formulation and In-Vitro Evaluation of Ciprofloxacin Loaded Topical Emulgel. International Journal of Pharmaceutical And Chemical Sciences, 2012; 1(1).
12. Dastidar DG and Sa B. A Comparative Study of Uv-Spectroscopy And First Order Derivative Methods For The Estimation Of Diazepam In Presence of Tween 20 And Propylene Glycol. AAPS Pharma Sci Tech, 2009; 10(4): 1396.
13. Kilor VA. Design And Development of Novel Microemulsion Based Topical Formulation of Hesperidin. Int J Pharm Pharm Sci, 7(12): 238-245.
14. Damle M and Vyawahare J. Development and validation of stability indicating HPLC method for determination of rutin and quercetin. The Pharma Review, September-October 2014: 113.
15. Kamble A, Adnaik R, Bhutkar M. Formulation And Evaluation Of Itraconazole Emulgel For Topical Drug Delivery. International Journal of Universal Pharmacy and Bio Sciences, 2014; 3(5): 32.