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Original Article

FORMULATION AND EVALUATION OF LIPOSOMAL GEL CONTAINING EXTRACT OF PIPRINE

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ABSTRACT

Objective: The objective of present research work is to develop Liposomes as a carrier system for 70% Hydroalcoholic extract, its incorporation in to gel formulations and to characterize the prepared and develop Liposomal gel formulation. There are many reports revealing the pharmacological potential of *Piper Nigrum.*

Methods: Cholestrol in various weight ratios were dissolved in 10 ml of Methanol: Chloroform (1:1) ratio used as a solvent. The extract solution was taken in a 500 ml round bottom flask. The flask was rotated in rotary flash evaporator at 40 rpm for 20 min in the thermostatically controlled water bath at 40 °C under vacuum 240 mmHg. The solvent was slowly removed by this process, and a very thin film of dry lipids was formed on the flask. The dry lipid film was slowly hydrated with 10 ml of Saline Phosphate Buffer pH 7.4 containing Insulin Drug. The flask was once again rotated at the same speed as before and at room temperature for 2 hr. The liposomal was left to overnight at 4°C, full lipid hydration.

Results: This study was done for herbal formulations used for topical delivery of therapeutic agents at the time of injury to accelerate skin repair in the shortest time possible, with minimal pain. Plant *Piper Nigrum.* Family Piperaceac is extensively used.

Conclusion: The present study revealed liposomal gel as an efficient carrier for herbal extract. Keywords: Piperine, Gel, Herbal extract, Liposomes, Liposomal gel.

Keywords: Liposomal gel, Containing extract of piprine

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INTRODUCTION [1, 2]

Liposomes have been investigated for many years as parenteral drug carrier systems, but only for approximately one decade, they have been considered for topical drug delivery, including ophthalmic and dermal treatments. With regard to the topical application, liposomes embedded in the topical dosage forms could provide a topical activity at the desired locus of action with little or no systemic activity. In general, they are deemed more effective and less toxic than conventional topical formulations, ointments, creams or lotions. Liposomal topical formulations may serve as a solubilizing matrix for poorly soluble drugs, penetration enhancers of the active ingredient into the skin, local depot (microreservoires) for sustained drug release as well as a rate-limiting membrane barriers for modulation of systemic absorption. In accordance to the abovementioned, local anesthetics together with anticancer, antifungal and antibiotic agents are among the substances whose incorporation into liposomes satisfied all the requirements necessary for topical application and localized drug delivery.

Vitiligo is known in Ayurveda as "*shwitra*". It is of twotypes, that is, *Kilas* and *Varuna*.

Medical and surgical treatments for vitiligo are suboptimal witheither poor response or continued the progression of vitiligo despite therapy. As far as medical therapies are considered, high potency topical steroids and narrowband ultraviolet irradiation are considered to be the effective form of monotherapy as per current evidence.

MATERIALS AND METHODS

Materials

Cholesterol, Methanol, Cabopol 940, PEG 400, Methylparaben, Proplyparaben and Cows Ghee, Chloroform, Dichloromethane were purchased from local market. The extraction was carried out by using dried seed of Black Paper In hydroalcohol solution.

Collection of seed material

The seed of Black Paper were collected from the Local market, Solapur, Dist.-Solapur, Maharashtra, India in Augest 2019, cleaned and used.

Authentication of plant

The seeds authenticated by Dr. M. N. Jagtap, HOD, Dept. of Botany, DBF Dayanand College of Arts and Science, Solapur. By comparing morphological features and a sample voucher of specimens having the cat. No. 9201

Preparation of extract [3]

100 gm of black pepper powder extracted with 1500 ml 95% ethanol in Soxhlet extractor for 2 h. The solution was filtered and concentrated on the water bath at 60 °C. 10 ml 10% of alcoholic potassium hydroxide was added to the filtrate with continuous stirring. The residue was filtered and alcoholic solution was left overnight and filtered through a membrane filter.

Calibration curve

10 mg of extract powder was accurately weighed and transferred to 10 ml clean and dry volumetric flask and phosphate buffer pH 5.8 was added in volumetric flask volume was adjusted to 10 ml to give the concentration of 1000µg/ml. from this 4 ml was pipetted out and transferred to another 10 ml clean and dry volumetric flask and volume was adjusted to 10 ml with phosphate buffer pH 5.8 to give the concentration of 400µg/ml. from this stock solution 0.5 ml,1 ml, 1.5 ml, 2 ml and 2.5 ml solution was pipetted out to give the concentration of 20,40,60,80 and 100µg/ml. the absorbance was measured at 243 nm and the calibration curve was plot.

Preparation of liposome by rotary flash evaporator method

Ghee: Cholestrol in various weight ratios were dissolved in 10 ml of Methanol: Chloroform (1:1) ratio used as solvent. The extract solution was taken in a 500 ml round bottom flask. The flask was rotated in rotary flash evaporator at 40 rpm for 20 min in thermostatically controlled water bath at 40 °C under vacuum 240 mmHg. The solvent was slowly removed by this process and very thin film of dry lipids was formed on the flask. The dry lipid film was slowly hydrated with 10 ml of Saline Phosphate Buffer pH 7.4 containing Insulin Drug. The flask was once again rotated at the

same speed as before and at room temperature for 2 h. The liposomal was left to overnight at 4 °C, full lipid hydration.

Preparation of liposomal gel [4]

The appropriate amount of Carbopol 940 was weighted and added slowly in a phosphate buffer solution (pH 5.8), under constant stirring by a paddle stirrer. After addition of solid material, the gel was allowed to swell under moderate stirring for at least 12 h. Or until fully swollen and transparent. Other ingredients, such as 15% w/v polyethylene glycol-400 (PEG-400) and triethanolamine (0.5% w/v), were added to obtain a homogeneous dispersion of gel and sodium benzoate (0.5% w/v) was added in the buffer used for gel preparation. Liposomal gel formulations were prepared by mixing the liposomal dispersions with the gels in the ratio of 1:5 (w/w) (liposome dispersion/gel).

Table 1: Formulation table

S. No.	Components	F1	F2	F3
1	Liposomes	Equivalent to 100 mg	Equivalent to 100 mg	Equivalent to 100 mg
2	Carbopol 940	0.5%	1%	1.5%
3	Triethanolamine	q. s	q. s	q. s
4	PEG400	2 ml	2 ml	2 ml
5	Methyl paraben	q. s	q. s	q. s
6	Propyl paraben	q. s	q. s	q. s
7	Water	q. s	q. s q. s	

Evaluation of prepared gels [5-8]

Physical evaluation

The formulations Liposomal gel was evaluated for organoleptic characteristics, occlusiveness and wash ability.

Measurement of pH

The pH of the formulated gels was determined using a digital pH meter. The electrode was immersed in the gel and readings were recorded from pH meter.

Viscosity study

Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. 30 gm of gel preparation was kept in 50 ml beaker, which was set till spindle groove was dipped and rpm was set and reading was measured after five minutes. Viscosity was calculated by using factor. The procedure was repeated three times and observations are recorded as mean.

Spreadability

A gel sample of 0.1 g of each formula was pressed between 2 slides and left for about 5 min. It's no more spreading was expected. The diameters of spreaded circles were measured in cm. It was taken as comparative values for spreadability.

S = ML/T

Where S = Spreadability, M = weight tied to upper slide, L = length of glass slide and T = time taken by the slide to separate from.

Extrudability study

The gel formulations were determined by filling gel in the collapsible tubes. The gel formulation was determined in terms of weight in grams required to extrude a 0.8 cm. ribbon of gel.

Skin irritation test

This test was performed on human volunteers. Three volunteers were chosen for single formulation and study was performed after taking their informed consent. It was applying gel on an area of 4 square inch to hand. Then the result for the irritation was done.

Drug content

Weighed 15 gm of each gel formulation were transferred in 100 ml of the volumetric flask containing 10 ml of alcohol and stirred for 35 min. 1 ml of the above solution diluted to 10 ml with alcohol and again, 1 ml of the solution was further diluted to 10 ml with alcohol. The absorbance of the solution was measured spectrophotometrically at 269 nm.

$$\text{Drug content} = \frac{\text{Absorbance}}{\text{Slope}} \times \text{Dilution factor} \times \frac{1}{100}$$

***In vitro* diffusion studies**

The drug release from the formulations was determined by using the apparatus, which consists of a cylindrical glass tube which was opened at both the ends. 1 gm of gel equivalent to 10 mg was spread uniformly on the surface of the cellophane membrane (previously soaked in the medium for 24 h) and was fixed to the one end of tube. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touches (1-2 mm deep) the surface of diffusion medium i.e. 100 ml of pH 5.8 phosphate buffer contained in 100 ml beaker, The assembly was placed on thermostatic hot plate with magnetic stirrer and maintained at temperature 37 °±2 °C the contents were stirred using magnetic bar at 100rpm for a period of 5 h, 5 ml of samples were withdrawn at different time intervals and replace with 5 ml of fresh buffer and after suitable dilution, the sample was analyzed at 269 nm.

Stability study

Stability studies of liposomal suspension and gel were done for one month under conditions required. Accelerated stability studies were performed by keeping the temperature 4°C and 38 °C. The stability was evaluated by comparing the physical examination, pH measurement, drug content and *In vitro* diffusion studies.

RESULTS AND DISCUSSION

Physical evaluation

The prepared Liposomal gel formulation was inspected visually for their colour and appearance. The developed formulations F1, F2, and F3. Were colorless. All the formulations were much clear.

Table 2: Evaluation of liposomal gel

S. No.	Batch	Physical evaluation	pH	Viscosity study (cps)	Spread ability (gm. cm/sec)	Extruded amount (%)
01	F1	Good	7.1	41560	12.08	74.15
02	F2	Good	6.9	62521	11.50	76.65
03	F3	Good	6.8	61586	11.52	78.46

Measurement of pH

The pH of the formulated gels was determined using a digital pH meter. The electrode was immersed in the gel and readings were recorded from pH meter. The results in table 2.

Viscosity study

Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. It show in table 2.

Spreadability

A gel sample of 0.1 g of each formula was pressed between two slides and left for about 5 min and no more spreading was expected. The diameters of spreaded circles were measured in cm. It was taken as values for spreadability. It shows in table 2

Extrudability study

The gel formulations were determined by filling gel in the collapsible tubes. It was determined in terms of weight in gm required to extrude a 0.5 cm. ribbon of gel. Result was shown in table 2.

Skin irritation test

This test was performed on human volunteers. Three volunteers were chosen for single formulation and study was performed after taking their informed consent. No skin irritation feel to any volunteers.

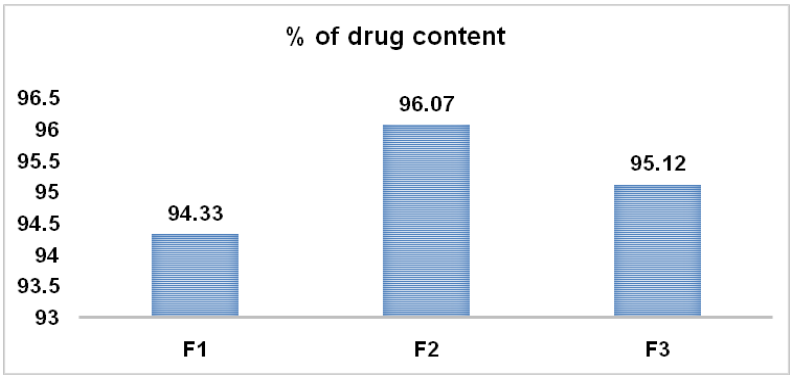

Fig. 1: % of drug content of liposomal gel

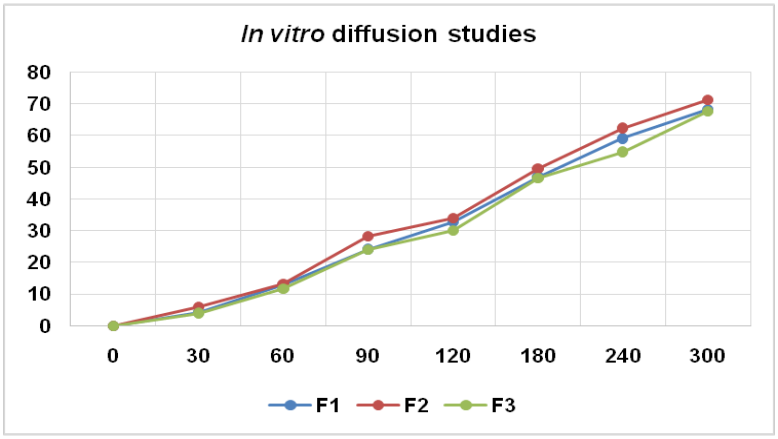

Fig. 2: *In vitro* diffusion studies of liposomal gel

Drug content

The drug content was shown in fig. 1.

***In vitro* diffusion studies**

The Liposomal gel formulation F1, F2 and F3 shows drug release68.19, 71.20 and 67.66% respectively up to 5 h.

Stability study

Stability studies of liposomal suspension and gel were done for one month under conditions required. The stability was evaluated by comparing the physical examination, pH measurement, drug content and *In vitro* diffusion studies. There is no change obtained during the stability study.

CONCLUSION

Various formulation (F1, F2 and F3) were developed by using a Carbopol 940. Developed formulations of Liposomal gel containing extract of *Piper Nigrum* were evaluated for the physiochemical parameters such as drug content, pH, viscosity, spreadability, extrudability, *in vitro* drug diffusion. Viscosity studies of various formulations revealed that formulation F2 was better to compare to others. The all the developed formulation, F2 shows better drug diffusion, did good Results. pH of the F2 formulation is sufficient enough to treat skin infections. Thus, Liposomal gels can be successfully prepared using Carbopol-940 as gelling agents suitable for topical application. Hence formulation F2 should be further developed for scale-up to industrial production.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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