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PREPARATION AND CHARACTERISATION OF CUMIN SEED OIL EXTRACT PHYTOSOMAL GEL

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Abstract:
Phytosomes containing vesicular drug delivery system wherein phytoconstituents of herb extract nearby and bound by lipid (one phyto-constituent molecule linked with at least one phospholipid molecule) Thymoquinone is the main active constituent of Cumin seed oil. Using Design of experiment 5 different formulations were prepared. Two different independent variables were used which include: Amount of lipids (F1), Amount of cholesterol (F2). All the formulations was prepared using thin film hydration method. The Thymoquinone was more soluble in Methanol than in Ethanol, phosphate buffer pH 7.4 and water. The prepared thymoquinone was subject to the %EE. The highest entrapment efficiency was found to be 90.8 ± 0.6 % which is selected as optimized formulation and evaluated for Homogenicity, spreadability, pH, Viscosity, Stability.

Keywords:- Thymoquinone, Phytosome, Phospholipid.

I. Introduction :

The name 'phyto' means plant where 'some' means cell-like. Phytosomes containing vesicular drug delivery system wherein phytoconstituents of herb extract surround and bound by lipid (one phyto-constituent molecule joined with at least one phospholipid molecule) fig no.1. The phospholipid molecular structure made up of a water-soluble head and two fat soluble tails, because of their double solubility, phospholipid acts as an effective emulsifier.[10] Black seed (also known as black cumin, Fennel Flower, Nutmeg Flower, Black seed, Black Caraway, Roman Coriander, Damascena, Devil in-the-bush, Wild Onion Seed.; Nigella sativa) is an yearly flowering plant belonging to the family Ranunculaceae and is a home grown of Southern Europe, North Africa, and Southwest Asia. Black cumin is cultured in the Middle Eastern Mediterranean region, Southern Europe, Northern India, Pakistan, Syria, Turkey, Iran, and Saudi Arabia. Nigella sativa contain many constituents like thymoquinone, Thymohydroquinone, dithymoquinone, Thymol, Protein, Fat, Carbohydrates, unsaturated fatty acids like linoleic acid, olic acid, saturated fatty acids like palmitic acid, steric acid etc. Thymoquinone (TQ) is the most large active constituent of the volatile oil of Nigella sativa seeds and most properties of N sativa are mainly assign to TQ. A number of pharmacological actions of TQ have been look over like antioxidant, antiinflammatory, immunomodulatory, anti-histaminic, anti-microbial, anti-tumor effect etc. It has also the properties to counteract the gastric mucosal damage, hepatoprotective, nephroprotective and Cytrostatic activities. In addition, beneficial impact of TQ in cardiovascular disarray, diabetes, reproductive disarray and respiratory ailments, as well as in the treatment of bone difficulty as well as fibrosis have been shown. Thymoquinone is in large quantities present in seeds of Nigella sativa that is popularly known as black cumin or black seed. A large number of studies have disclose that thymoquinone is the major active constituent in N. sativa oil this constituent is in control of the majority of the pharmacological properties. Thymoquinone has also been shown to alter many molecular and signaling pathways in many inflammatory and degenerative diseases including cancer. In addition, a more data shows that TQ has very low negative effect and no serious toxicity. Hence the present study was used to formulate, improve and evaluate the phytosomal gel of thymoquinone in different percentages (0.4% phytosomal gel of thymoquinone)

II. Materials and Methods

Materials:

Thymoquinone was obtained from Sigma Aldrich, soya lecithin was obtained from Himedia, Mumbai, India, cholesterol, Methanol, Dichloromethane, triethanolamine was obtained from Fischer Scientifics, Mumbai, India. Potassium dihydrogen phosphate and sodium hydroxide abd Carbopol 934 was procured from S.D fine chemicals.

Mehtods:

- **Preparation of Thymoquinone loaded phytosomes:**

The Thymoquinone loaded phytosomes are prepared by using Thin film method. Accurate amounts of Phospholipids (X1), Cholesterol(X2) (shown in table no.1) and drug were dissolved in a mixture of organic solvents consisting of Dichloromethane: Methanol (2:1, v/v) in a dry, round-bottom flask. The organic solvent was allowed to evaporate using

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a rotary evaporator (Aditiya scientific evaporator) adjusted to 60 rpm, at 40° C for 15 mins under low pressure to prepare a thin lipid film on the wall of the roundbottom flask. The dry thin lipid film was subjected to hydration with phosphate-buffered saline (pH 7.4) by rotation for 1 h at 60 rpm at room temperature. The multilamellar lipid vesicles (MLVs) were then sonicated using the ultrasonic probe Sonicator (Mangaldeep tech solutions) for 30 min to reduce the vesicle size and stored at 4°C for further investigation.(1)

- **Preparation of thymoquinone phytosomal gel**

0.2% and 0.4% of thymoquinone phytosomal gel was prepared.The accurate weight of polymer was sprinkled into a beaker containing 60 mL boiling distilled water, and then soaking was allowed overnight. The Phytosomal dispersion containing 0.2% and 0.4% TQ was added with continuous stirring to allow homogeneous distribution of TQ Phytosomes within the gel base. Sodium benzoate is used as preservatives. The dispersion was neutralized by addition of Triethanolamine dropwise, with continuous mixing until a homogenous gel was obtained Prepared gels were stored in suitable containers at room temperature for further studies.(2)

Characterisation of Thymoquinone phytosome

- Determination of Entrapment Efficiency (EE %) 5 ml of Thymoquinone phytosomal complex was added to phosphate buffer pH 7.4 and were centrifuged at 4000 rpm for 45 min 40C to allow the separation the entrapped drug from the un-entrapped drug using a Remi ultra centrifuge (Remi-CM12plus). Results in formation of sediment and supernant. From this unentrapped drug is separate by removing the supernant then the sediment was lysed with methanol and then analyzed at 254nm using UV-Visible spectrophotometer (Elico, PG instruments). The percentage drug entrapment was calculated by using the formula.(3)

EE% = [Amount of Entrapped TQ/Total Amount of TQ] /100

- **Determination of Particle Size :**

Particle size were measured for all prepared TQ phytosomes using the dynamic light scattering (DLS) technique at 25°C using the Particle Size System. (4)

Characterisation of Thymoquinone phytosomal Gel

- **Homogenicity:**

Homogeneity of the developed gels were tested for by visual inspection by pressing small quantity of both gel (0.2% and 0.4% Thymoquinone phytosomal gels) between the thumb and the index finger. They were tested for their appearance and presence of any aggregates. The consistency was determined as homogeneous or not.(5)

- **Spreadability :**

Two glass slides of 20 cm × 20 cm were selected. A small amount of gels was sandwiched between the two glass slides. A 500 g weight was placed on the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and then fixed to a stand without slightest disturbance in such a way that the upper slide slides off freely, to the force of weight tied to it. The time taken for the upper slide to separate away from the lower one was noted using a stop clock. (5)

The following equation was used for this purpose: $S = m \times L/T$

- **Viscosity:**

Viscosity of both the formulated gels was measured using Brookfield viscometer (Brookfield DV-E viscometer). using spindle number S64 rotated at a speed of 12 rpm for a 10-s run time at 37°C. (5)

- **Measurement of pH:**

One gram of both the gels was dispersed in 20 mL of distilled water, and a digital pH meter (Systronics Digital - 335) was used to determine the pH value. The measurement was performed three times and the mean ± SD was calculated.(5)

- **Drug content:**

1gm of each gel was dissolved in 100 ml volumetric flask by using methanol. This was then stirred for 2 h. The resultant solution was filtered, and drug content was analyzed spectrophotometrically at 254nm. (5)

Stability studies:

The stability studies of TQ Phytosomal gel (0.2% and 0.4%) were conducted at refrigerated temperature (4°C) and room temperature as per Guidelines of International Conference on Harmonization (ICH). Samples were analyzed for physical appearance drug content, and in vitro diffusion studies after 15, 30 and 45 days. (6)

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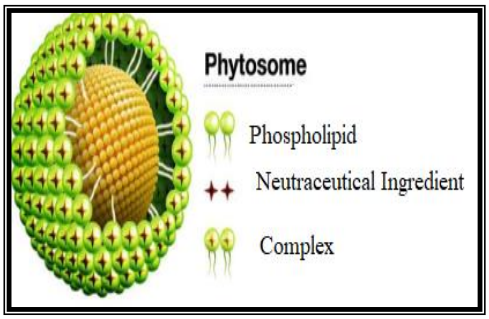

Fig No 1 : Structure of Phytosome

Factors	Low level	High level
X1 - Amount of lipid (mg)	550	850
X2 - Amount of Cholesterol (mg)	62.2	87.5

Table No. 1 : Formulation factors for multilevel factorial design

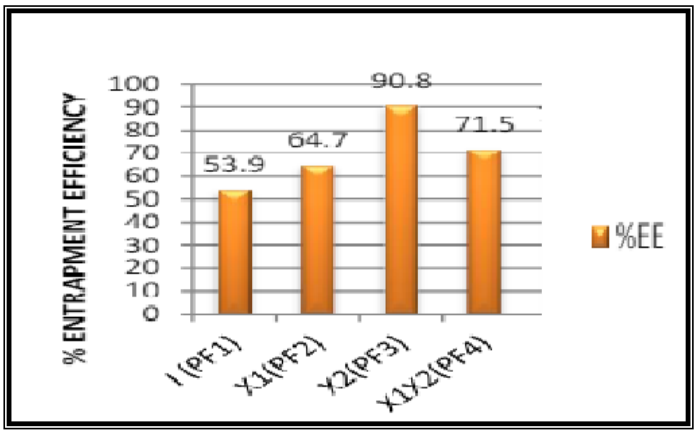

Table No. 2 : Entrappment Efficiency for all TQ Phytosomal Formulation formulation

DURATION	4±2⁰ C	
	DC	%CDR
INITIAL	95.5±1.25	96.6.±0.42
15 days	96.89±1.01	97.4±1.5
30 days	96.02±0.21	97.1±0.21
45 days	94.53±1.22	98.1±1.41

Table No. 3 Stability of 0.4% Thymoquinone phytosomal gel 4+/-2^{0}C

Evaluation	0.4 % phytosomal gel
Homogenecity	Good
Spreadibility	72.5 ± 0.52
Viscosity (cps)	17291 ± 0.25
pH measurement	6.5 ± 0.58

Table No 4 : Evaluation of 0.4 % TQ Phytosomal gel

III.Result and Discussion

- **Entrapment efficiency:**

As shown in Table 2, it was found that the prepared Thymoquinone phytosomes exhibited a good EE%, with values ranging from (50.7±0.2) for (PF1) to (90.8±0.6%) for X2 (PF3).

- **Particle size Prepared TQ phytosomes:**

The prepared TQ phytosomes were tested for particle size and zeta potential. From the results of particle size, it was found that all prepared TQ phytosomes have a particle size less than 200 nm, and as such are effective for transdermal applications.

• **Evaluations of Thymoquinone phytosomal gel:**
0.4% thymoquinone phytosomal gel: TQ phytosomal gel is smooth with a homogenous appearance. The Spreadability values is 72.5±0.52cm, respectively, which indicates that they can be spared easily on skin surface with little stress. The viscosity of 0.4% Thymoquinone phytosomal gel is found to 17291±0.25 cps. The pH value is found to be 6.5±0.58 which is considered within the normal range of pH for topical preparations.(see table 4)

• **Stability studies**
Stability of 0.4% thymoquinone phytosomal gel At 4 ± 2^0C shown in table 3. DC- Drug Content, C.D.R- Cumulative Drug Release Stability studies showed that Phytosomal gel is more stable at 4℃ when compare to other temperatures. There was a change in color for the samples kept at room temperature, leakage of drug was minimum due to gel formulation because it is viscous in nature and also decreases the fusion of vesicles which otherwise will be responsible for drug leakage.

Discussion :
The aim of the study was to Preparation amd Characterisation of Cumin seed oil extract phytosomal Gel. TQ phytosomes was prepared by Rotary evaporation technique (thin film method) and formulations was designed by using design expert software(table no.1) so according to the design of experiment 4 formulations was prepared. Independent variables used were phospholipid and cholesterol as X1 and X2 and dependent variables as entrapment efficiency, and particle size. The concentration of phospholipid was responsible for the drug release and the concentration of cholesterol is responsible for the entrapment efficiency. 0.4% thymoquinone Phytosomal gels was prepared of optimized formulation and subjected for different evaluations like homogenicity, spreadability, pH and viscosity(table 4). Stability studies were also performed for the 0.4% thymoquinone phytosoma gel. Stability studies showed that Phytosomal gel is more stable at 4℃ when compare to room temperatures. 0.4% of the gel shows higher stability compare to 0.2% thymoquinone phytosomal gel (table 7 & 8). There was a change in color for the samples kept at room temperature, leakage of drug was minimum due to gel formulation because it is viscous in nature and also decreases the fusion of vesicles which otherwise will be responsible for drug leakage.

Conclusion:
The aim of the study to enhance the permeability by formulating the Thymoquinone loaded Phytosomal gel. Thymoquinone has use to treat diabetes, cancer, wound healing, bacterial infection even in diabetic wound. Preformulation studies shows high solubility in methanol and FTIR shows no interaction between drug and polymer, it was concluded that the factorial design had the ability to obtain an optimized formula of Thymoquinone phytosomes, with high EE% (90.8 ± 0.6), small particle size (156± 0.02). After optimization of formulation variables, it was found that the optimized formulation was suggested to contain 550 and 87.5 mg of X1 and X2, respectively. Incorporation of optimize Phytosomes formulation into gel, The concentration of Thymoquinone in the prepared Phytosomal gel was 0.4% w/w. 0.4% was considered to be optimized gel based on evaluations. 0.4% gek shows The Spreadability values 72±0.52 cm, which indicates that they can be spared easily on skin surface with little stress. The pH value is found to be 6.5±0.58 which is considered within the normal range of pH for topical preparations. The actual drug content of the Thymoquinone phytosomal gel was found to be 95.5±1.29%, which represents good content uniformity. The viscosity of Thymoquinone phytosomal gel is found to 17291±0.25cps.

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REFERENCES:
1. Ashwini s dhase and shweta saboo, preparation and evaluation of phytosomes containing methanolic extract of leaves of Aegle Marmelos (Bael), international journal of pharmatech research. 2015, 8(6), 231-240.
2. Rudra Pratap Singh and Ramakant Narke, preparation and evaluation of phytosome of lawsone, international journal of pharmaceutical sciences and research. 2015, 6(12): 5217-5226.
3. Solmaz resale, saeed ghanbarzadeh, maryam mohammadi, hamed hamishehkar, Nano phytosomes of quercetin, A promising formulation for fortification of food products with antioxidants, journal of pharmaceutical sciences. 2014, 20, 96-101
4. Srinivas, Development of A Topical Phytosomal Gel of Woodfordia Fruticosa, World journal of pharmacy and pharmaceutical sciences. 2015,4(11):919-931
5. Julie mariam joshua, athira anilkumar, verjina cu, deepa t vasudevan, saritha a surendran, Formulation and evaluation of antiaging phytosomal gel. 2018, 11(3), 409-422
6. Ahmed n allam, Ibrahim a komeil, ossama y abdallah, Curcumin phytosomal gel formulation: Development, optimization and physicochemical characterization, acta pharm (research gate). 2015, 65, 285-297

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7. Prasuna Sundari Pingali, Prathima Srinivas, B. Madhava Reddy: Miconazole Loaded Novel Phytosomal Topical Gels, World journal of pharmacy and pharmaceutical sciences. 2015,4(10):2305-2320
8. K. Rajashekar, P. J. Prasuna Sundari, Dr. Prathima Srinivas, Development of A Topical Phytosomal Gel of Woodfordia Fruticosa, World journal of pharmacy and pharmaceutical sciences. 2015,4(11):919-931
9. Malay k das and bhupen kalita, design and evaluation of phyto- phospholipid complexes (Phytosomes) of rutin for transdermal application, journal of applied pharmaceutical sciences. 2014, 4(10), 051-057
10. Harshal ashok pawar and bhagyashree dilip bhangale, Phytosome as a novel biomedicine: A micoencapsilated drug delivery system, journal of bioanalysis and biomedicine. 2015, 7(1), 006-012