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FORMULATION DEVELOPMENT AND EVALUATION OF SILYMARIN GEL FOR PSORIASIS TREATMENT

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ABSTRACT

The present work represents topical gel formulation of Silymarin obtained from the seeds of plant *Silybum marianum* (L.) Silymarin is mixture of flavolignan. It consists of silybinin, silydianin, silychristin and isosilybinin. Silymarin is used as Hepatoprotective from ancient time apart from it is used as antioxidant, anti-inflammatory, anti-fibrotic, immunomodulatory and liver regenerating action. It also used clinically in liver disease, viral hepatitis, amanita mushroom poisoning, neuroprotective and neurotropic action. The various formulation of silymarin is available like tablet, capsule, suspension, syrup in market. The present aim of the study to develop silymarin gel and check antipsoriasis activity. By using methanol as co-solvent and the Carbopol is used as gel forming agent and methyl Parabenand Propyl Paraben as preservative. The glycerin as humectants and Tween 80 used as surfactant. Gel are subjected to various physicochemical parameters such as pH, drug content, spreadability, viscosity, in vitro drug release was carried out in phosphate buffer (pH 6), primary skin irritation test and antifungal activity was checked. The stability study was done as per the ICH guidelines. The drug content, pH, spreadability of the formulation was found to be 96.6%, 6.8, 25.45gm.cm/sec respectively. From the rheogram it is concluded that gel shows pseudoplastic flow property. It shows maximum drug release 96.30% over a period of 3h.The formulation did not show acute skin irritancy.

INTRODUCTION

Silymarin obtained from *Silybum marianum*, commonly known as 'milk thistle' (Family: Asteraceae/Compositae).It is an annual and biannual herb, stem 20-150 cm high, green, glabrous. Leaves alternate, large, glossy, green, white veined, glabrous with strongly spiny margins, basal leaves (25-50 cm long, 12-25 cm wide) cauline, pinnatifid. Fruits 6-7 mm long composed of 6-8 hard skinned achenes with a white, silky pappus (15-20 mm in diameter). Milk Thistle was named silybumby Dioscorides in 100 A.D. Milk Thistle contains 70 to 80% flavolignan and 20 to 30% unidentified fraction. Milk Thistle contains silybin, silydianin and silychristin collectively known as silymarin. Silymarin is used for liver disease more than 2000 years. It is used as chemoprotectiveand anticancer agents, adjuvant therapy of cancer, Neuroprotective and neurotropic activity, treatment and prevention of gastrointestinal problems, nephropathy and cardio-pulmonary problems. In skin protection.

MATERIALS AND METHODS

Silymarin obtained from M. J. Biopharm. Pvt, Ltd. Taloja, Navi-Mumbai.Methyl Paraben and Propyl Paraben from LobaChemiePvt Ltd. Mumbai.Glycerin from QualigensFine Chemicals Mumbai. And all other chemicals used are of analytical grade.

Preparation of formulation:

The 1 % w/w Silymarin was taken for the preparation of thegel. Methanol is used as co-solvent and as a dispersionmedium for silymarin. Weigh accurate amount of Carbopoland dispersed in sufficient amount of water. Then glycerinis added to it. And adjust the pH by addition of Triethanolamine between 6.8 to 7.4.The required quantity of silymarin was dissolved in small amount of methanol, then Propyl Paraben and methyl Paraben is added in it. Then it added to the formulation. Then add Tween 80 to it.

Table No. 1: Formula of the gel

Sr.No.	Ingredients	Quantity(gm)
1	Silymarin	1.3gm
2	Carbopol	0.5gm
3	Glycerin	10ml
4	Triethanolamine	0.5ml
5	Methyl Paraben	0.18gm
6	Propyl Paraben	0.05gm
7	Tween 80	2ml
8	Methanol	10ml
9	Water	q.s to 100gm
Total		**100gm**

Evaluation of Gel:

The gel was evaluated for the Ph, drug content, viscosity, spreadability, drug diffusion, stability studies, antifungal activity and primary skin irritation test conducted on human healthy volunteers.

Determination of pH:

Weigh accurately about 5gm of gel in 100 ml beaker add 45 ml of water disperse the gel in it. And determine the pH of solution at 27 0 C by using digital pH meter.

Drug content:

Weigh the quantity of the formulation equivalent to 100 mg of silymarin in 100 ml volumetric flask. Add 10ml of water to it and shake for 10min then add 70 ml methanol to it and sonicate for 10 min and adjust the volume to 100 ml. Pipette out 2ml form it dilute to 100 ml with phosphate buffer pH 7.5. Filter through the Whatman filter paper 42 and measure the absorbance using UV spectrophotometer at 286nm.

Viscosity:

Viscosity of the gel performed by using Brookfield Viscometer (using model CAP2002+2) using spindle cp-52.At varying speed and shear rate. The measurement was done at a speed of 10, 20, 30, 40, 50 rpm. The viscosity was determined at room temperature.

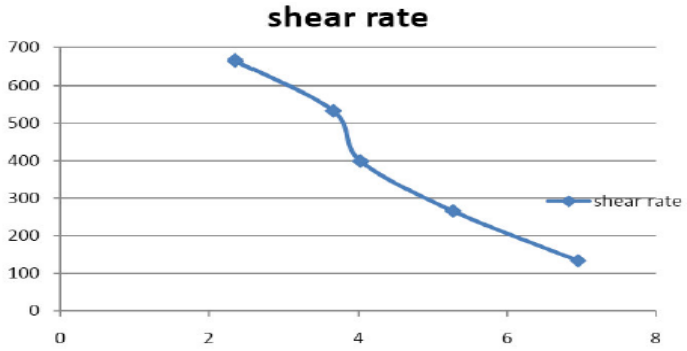

Fig No. 1 Viscosity Vs Shear rate graph for formulation

Spreadability:

For the determination ofSpreadability excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 mins. Weight of 50 g was added to pan the time required to separate the 2 slides, i.e. the length in which the upper glass slide moves over the lower plate was taken as measure of spreadability(S).

$$S = ml/t.$$

Where, m - Weight tide to upper slide
l - Length moved on the glass slide
t - Time of movement

***In vitro* Drug Diffusion study:**

A Keshary-Chien (K-C) diffusion cell with a receiver compartment volume of 27 ml, diameter of 2.6 cm and effective diffusion area of 5.31cm2 was used in this study. Synthetic membrane (cellophane) was used as diffusionmembrane. The synthetic membrane (cellophane) was soaked for 12 hours in phosphate buffer (pH 6) before subjecting to diffusion study. The membrane was positioned between the two cell halves of a glass chamber.The two compartments were held together with a clamp. The lower receptor compartment contained 25ml ofphosphate buffer (pH 6) and in the upper donor compartment 1gm of gel was spread, evenly on themembrane. The receptor phase (phosphate buffer pH 6) was continuously stirred with help of magnetic stirrer and was maintained at temperature of 37 ±1oC during the experiments. At 30, 60, 90, 120, 150, 180 mins timeintervals, 3 ml (or so) of the sample was withdrawn from the receiver compartment and the same amount of freshbuffer solution was added to maintain the sink conditions in receptor compartment. The care was taken to ensure that no air bubbles were lodged underneath the diffusion membrane during the experiments. The samples were diluted up to 10ml with phosphate buffer pH 6 and were analyzed spectrophotometrically at a wavelength of 286 nm and the concentration of extract in each sample was determined from a previously prepared standard curve. This experiment was carried out for a period of 4 hours and in duplicate for each formulation.

Release Profile

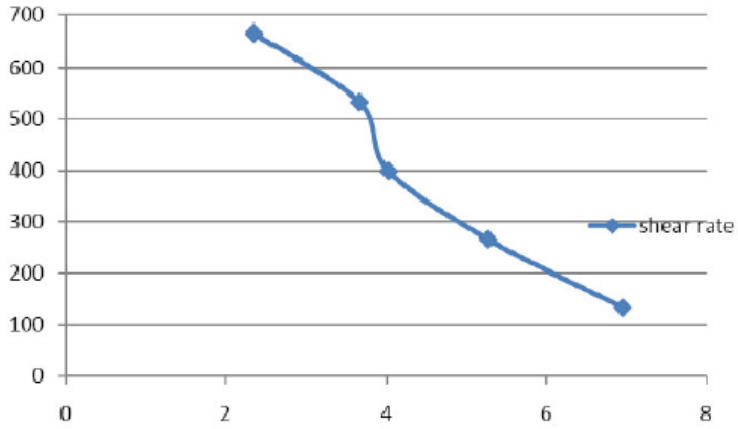

Fig No. 2 Drug release profile for formulation

Evaluation of antipsoriasis activity (Antifungal activity was checked):

Agar diffusion test:

Using the micropipette, 100 micro liter of 1 Macfarlandsolution of *candida albicans culture* (in SBD) was spreadover the surface of an agar plate using the sterile hockey stick. The same

procedure was followed for *C. Krusei* and *C. neoformans*. Using the sterile 5 cm plastic pipette, four holes were punched (2 mm in diameter) in each of the culture plate. In the first hole, 10 micro liter of the drug was added as positive control:10 micro liter of DMSO was added as a negative control in a second hole 5 and 10 micro liter of the plant extract were added in the third holes. The culture plates were then incubated at 370C and the results were observed after 24 hours. The clear zone around the plant extract was measured in mm and indicated the activity of the plant extract against the fungal organism. The experiments were done in triplicate.

Micro dilution assay:

The micro dilution method was employed to determine the minimum inhibitory concentration (MIC) of the plant extract using 96 wells micro titration plates as previously described. Briefly 185miro liter of the broth added into each well in the first row of the micro titration plate and 100 micro liters to the rest of the wells from the second row downwards. 15 of the plant extract positive control (Filucanazole) followed by negative control (the 20 % DMSO used to dissolve the plant extract) and the plant extract in the rest of the well on that row. A twofold serial dilution was done by mixing the contents n the each well of the first row and transferring 100 micro liters to the second well of the column and same then was done up to the last well of the column and the last 100 1from the last well was discarded. Then 100 of yeast suspension were added. The result was observed after 24 h. incubation at 370 C followed by the addition o f the 40 l of a 0.2 % Odo Nitro Tetrazolium (INT) solution after a further incubation of 4 hr. The wells that did not show any colorchange after INT was added indicating the concentration of the plant extract that was able to inhibit fungal growth whereas the pink color change indicated fungal growth.

Stability studies:

The prepared gel formulation were subjected to stability studies for three months as per the ICH norms at a temperature (40 ± 10C) The gel (50gm) was filled in wide mouth plastic bottles and placed in stability chamber. These samples were evaluated for appearance, pH and viscosity and observations were noted.

Table No. 2: Stability study of the gel.

Sr.No.	Time Interval (days)	Appearance	pH	Spreadability (gm.cm/sec)	Viscosity	Drug Content	Drug Release
1	15	Yellow	6.8	25.50	9.127	98.23%	96.60%
2	30	Yellow	6.7	24.35	9.542	97.56%	95.23%
3	45	Yellow	6.7	24.65	8.947	96.98%	96.45%
4	60	Yellow	6.6	25.01	9.174	97.54%	98.56%

Primary skin irritation test:

Healthy Human Volunteers study:

The study was performed on (2 male and 1 female) healthy human volunteers in the age 20-25 years, after prescreening them for any skin infections. It was confirmed that they had not received any anti-allergic medication for at least a month prior to the study. The test is performed to check any alteration in skin after application of the formulation. This test was carried by placing the gel on forearm with the help of cotton fabric (2-3sq.cm).This was then covered with a piece of uncoated cellophane about 5 sq.cm. And sealed to the skin, with a piece of adhesive plaster. The patches should not be exposed to sunlight or other sources of UV light. After 72 hrs, the patches are removed and an initial reading is taken one hour later. The final reading is taken a further 72 hrs later. The skin irritation evaluated by questioning the human volunteers after regular interval of time.

RESULT AND DISCUSSION

Silymarin is drug of choice in treatment of hepatic disorders. Various parameters are used for the evaluation of the gel. Silymarin gel shows yellow in color and percentage drug content in the formulation was found to be 98.20 %. pH of the silymarin gel shows 6.5 pH which is near towards neutral. Spreadability of the gel was found to be 25.50gm.cm/sec. Viscosity of the formulation checks at varying speed and shear rate. It shows the pseudoplastic flow property. Silymarin gel shows the maximum drug release within three hours. It shows 96.30 % release within three hours. While gel shows the minimum inhibitory concentration 5mg/ml against *Candida albicans*. The antifungal activity is compared with Fluconazole as standard it shows zone of inhibition nearer to the standard i.e. Silymarin gel shows 10mm zone while the Flucanazoleshows 15mm zone. During the stability study gel shows no significant changes in the formulation. In Appearance, Viscosity, pH, Spreadability, Percentage drug content and drug release. The silymarin gel shows no primary skin irritation after 72 hrs.

CONCLUSION

Topical formulations are widely accepted because it is having effective and easy to administer. Silymarin gel shows the good viscosity which shows the pseudoplastic flow property. Gel shows good spreadability and pH lie in the range of skin pH. It has been observed that gel shows good antifungal activity like Fluconazole. Formulation shows the stability up to two month at the temp.400c. And it shows no skin irritation in human volunteers.

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