International Journal of Advances in Pharmaceutics
E-ISSN: 2320-4923; P-ISSN: 2320-4931
Journal DOI: https://doi.org/10.7439/ijap
Journal home page: https://ssjournals.com/index.php/ijap **Research Article**

Formulation, development, and evaluation of Silymarin loaded topical gel for fungal infection

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Abstract

The objective of work was the formulation of Silymarin Loaded Topical gel obtained from the seeds of plant *Silybum Marianum* (L). Silymarin is a composition of flavolignan. It includes Silibinin, Silydianin, Silychristin, and isosilybinin. silymarin is used as Hepatoprotective, Antioxidant, Anticancer, Anti-Inflammatory, Anti-fibrotic, Immunomodulatory a Liver Regenerating action. It also used in viral hepatitis neuroprotective and Neurotropic action. The different type of formulation available in tablet, capsule, syrup, suspension, nanoparticles in the market. The aim of the study was to formulate and develop silymarin Loaded gel and to check its Antipsoriasis activity, with the use of Methanol as co-solvent the HPMC as Gel forming Agent and Methyl paraben and propyl paraben as Preservative. The glycerin acts as Humectant and Tween 80 used as a surfactant. The Characterization of gel such as pH, Drug content, spreadability, viscosity, *in-vitro* drug release was carried out in pH 6, Primary skin irritation test and antifungal activity were checked. The drug content was found to be 95.8%. Spreadability of the gel was found to be 20.66gm cm/sec. The pH of silymarin loaded gel was found to be 6.9. The silymarin loaded gel shows the pseudoplastic flow from the rheogram. It shows Drug release 96.08% over a period of 3h. There was no acute skin irritancy found.

Keywords: Silymarin, Topical Gel, Antipsoriasis Activity, Antifungal activity.

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***Article History:**
Received: 15/01/2019
Revised: 21/04/2019
Accepted: 01/06/2019
DOI: https://doi.org/10.7439/ijap.v8i1.5041

QR Code

How to cite: Khedekar Y. B, Mojad A. A, Malsane P. N. Formulation, development and evaluation of Silymarin loaded topical gel for fungal infection. *International Journal of Advances in Pharmaceutics* 2019; 08(01): e5041. DOI: 10.7439/ijap.v8i1.5041
Available from: https://ssjournals.com/index.php/ijap/article/view/5041

1. Introduction

Silymarin Obtained from Milk Thistle commonly known as *Silybum mariamum*. it belongs to the family of Astraceae. leaves are large, glossy, white veined and strongly spiny margine. The composition of Milk thistle is Silybin, silydianin, and Silychristin collectively known as Silymarin.its is used as a chemoprotective and anticancer agent, hepatoprotective, Neuroprotective and Neurotropic activity, treatment and prevention of Gastrointestinal problems, Nephropathy, and cardio-Pulmonary problem. It is also used in skin protection. [1]

2. Materials and methods

The silymarin was obtained as a gift sample from Alkem Laboratories Limited, Haryana. Glycerin was obtained from SD fine, Nashik. Propyl paraben and Methyl paraben was obtained from Loba Chemie Pvt.Ltd. Mumbai.

2.1 Preparation of Formulation

The accurate weight of silymarin 1.3 gm (1% w/w) taken for the preparation of gel. The co-solvent is used as methanol and as a dispersion medium for silymarin. Weigh an accurate amount of HPMC K 100 as a gel-forming agent and dispersed an insufficient amount of water. Then glycerin is added to it and adjusts the pH by addition of triethanolamine between 6.8 to 7.4. The Silymarin is dissolved in a small amount of methanol, then add the preservatives in it. Then this solution was added to the formulation. Ultimately Tween 80 was added to it. [2] Table 1.

Table 1: Ingredients of Formulations

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

3. Characterization of silymarin gel

3.1 Determination of pH

Accurately weight about 10mg of gel in 100 ml of the beaker and add 50 ml water and disperse the gel and determine the pH of the solution at 27^0C by using Digital pH meter. [6]

3.2 Drug content

Equivalent to 100 mg of silymarin gel formulation in 100 ml volumetric flask. Add 10 ml of water to it and shake for 10 min, then add 70ml methanol to it and sonicate for 10 min and adjust the volume to 100ml. pipette out 2ml front it dilutes with 100ml with phosphate buffer pH 7.4, then filter through the Whatman filter paper and determines the absorbance using UV spectrophotometer at 287nm. [6]

3.3 Viscosity

The viscosity of the gel was Determined by Brookfield Viscometer (Using Model CAP2002+2) using Spindle cp-52. at varying speed and shear rate. The measurement of intervals was done at speed of 10, 20, 30, 40 and 50 rpm. It was determined at room temperature. The viscosity results are shown in figure 1.[3]

3.3 Spreadability

Excess of sample is required to determine spreadability, it was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight of 50gm was added to pan the time required to separate the two slides that is the length in which the upper glass slide move over the lower plate was taken as measurement of spreadability (S). [4]

S= ml/t

Where, m- weight holds to upper slide; L-length moved on glass slide; t-time of movement

3.4 *In-Vitro* Diffusion study

The *in vitro* Release of drug from the Na Formulations was determined Using Membrane diffusion Technique by using 100 µm cellophane membranes. Silymarin Loaded gel equivalent to 10mg of silymarin from Formulation took and suspended in 100ml of buffer pH 6. the cellophane membrane was soaked for 12 hrs 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 receptor compartment contained 100 ml phosphate buffer pH 6 and the upper donor compartment contained gel was spread. The receptor phase was continuously stirred with the help of magnetic stirrer and was maintained at temp 37^0C at 30, 60, 90, 120, 150, 180 mins time intervals, 3 ml of sample was withdrawn from the receiver compartment and the same amount of fresh solution was added to maintained sink condition in the receptor compartment. The sample was Diluted up to 10ml with phosphate buffer pH 6 and was determined by UV spectrophotometrically at wavelength. this experiment was carried out for a period of 4 hrs and in triplicate for each formulation. [6] The release profile is shown in fig. 2 & 3.

3.5 Evaluation of Antifungal activity

3.5.1 Agar Diffusion test

Using Micropipette, 100 microliters of 1 Macfarland solution of *Candida albicans* culture (in SBD) was spread over 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 in each of the culture plates. In the first hole, 10 microliters of the drug were added as a positive control, 10 microliter DMSO was added as a negative control in a second hole 5 and 10 microliters of the plant extract were added in the third hole. The culture plates were then incubated at 37^0C and results were observed after 24 hrs. Then their indicating activities of the plant extract against the fungal organism, the experiment were done in triplicate. It is shown in figure 4. [6]

3.5.2 Micro Dilution assay

It is used to determine the minimum inhibitory concentration (MIC) of the plant extract using 96 wells microtitration plates as previously described. Briefly, 185 microliters of the both added into each well in the first row of the microtitration plate and 100 microliters to the rest of the wells from the second row downwards. 15 of the plant extract positive control (Flucanazole) followed by negative control and the plant extract in the rest of the row. For the mixing, a twofold serial dilution was done by mixing the contents and each well of the rest row transferring 100 microliters to the second well of the column and same then was done up to the last well of the column and 100 lit from the last was discarded. After the yeast suspension was added. The result was observed after 24 hrs. incubation at 37^0C followed by the addition of the 40 lit of a 0.2 % Odo Nitro Tetrazolium (INT) After incubation of 4 hr.the result shows that there was no color change after INT was added indicating the conc. of plant extract that was able to inhibit fungal growth whereas the pink color change indicated fungal growth. [6]

3.6 Stability study

The formulated gel was subjected to stability studies for three months as per the ICH norms at a temp . (40 ± 10℃). The gel was filled in wide mouth plastic bottles and placed in the stability chamber. These samples were evaluated for pH, Appearance and viscosity were noted. The results are shown in table 2.[6]

3.7 Primary Skin irritation test

Healthy Human Volunteers study

The study was performed on healthy volunteers in the age between 20-25 years, after prescreening them for skin Infections.

Conclusion of this study 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 the forearm with the help of cotton fabric. 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. after 72 hrs, the patches are removed and initial reading was taken one hour later. The final reading was taken a further 72hrs later. The skin irritation evaluated by human volunteers. [7]

4. Result and discussion

Silymarin was used as a hepatoprotective and anticancer agent. The various parameter is used for the characterization of gel. Silymarin gel shows yellow color and percentage drug content in the formulation was found to be 95.20%. The pH of the silymarin gel was found to be 6.8 which was near toward neutral. Spreadability of the gel was found to be 25.50gm.cm/sec. The viscosity of the formulation checks at varying speed and shear rate. It shows a pseudoplastic property. It shows the maximum drug release within three hrs. It shows 96.30% release within 3 hrs. While gel shows the minimum inhibitory concentration 5mg/ml against Candida albicans.The antifungal activity was compared with fluconazole as standard and silymarin gel shows 10mm zone with fluconazole shows 15mm zone. During stability, study gel shows no significant changes in the formulation. The silymarin gel shows no primary skin irritation after 72 hrs.

Table 2: Evaluation of Formulated gel

Sr. No.	Time intervals (Days)	Appearance	pH	Spreadability (gm.cm/sec)	Viscocity	Drug Content (%)	Drug release (%)
1	15	Yellow	6.6	24.01	8.942	97.56	96.60
2	30	Yellow	6.8	25.50	9.145	98.25	96.45
3	45	Yellow	6.7	24.08	9.456	94.65	98.26
4	60	Yellow	6.7	26.01	8.125	99.23	96.45

Fig. 1: Viscosity Vs Shear rate

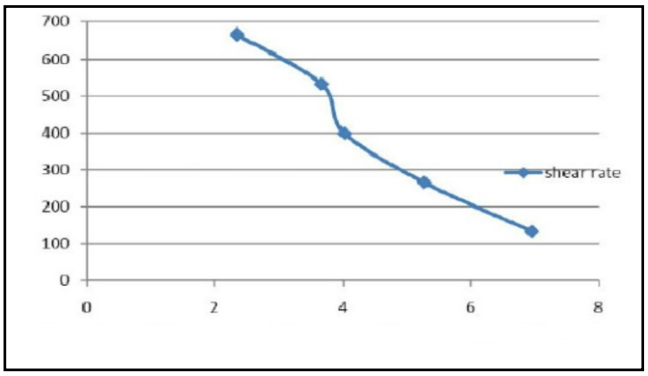


Fig. 2: Drug Release Profile of Formulation

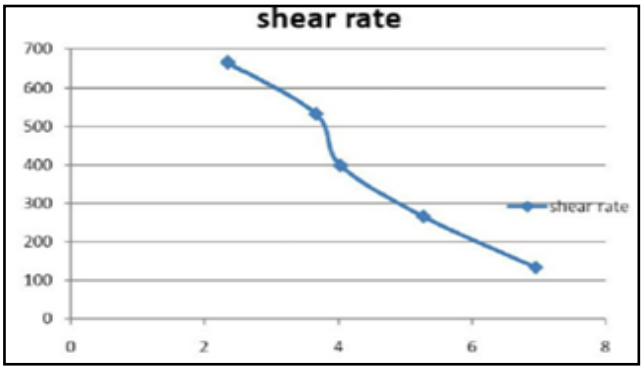


Fig. 3: *In- Vitro* Diffusion Study

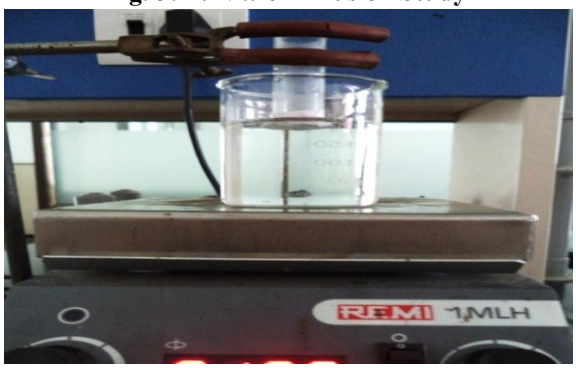
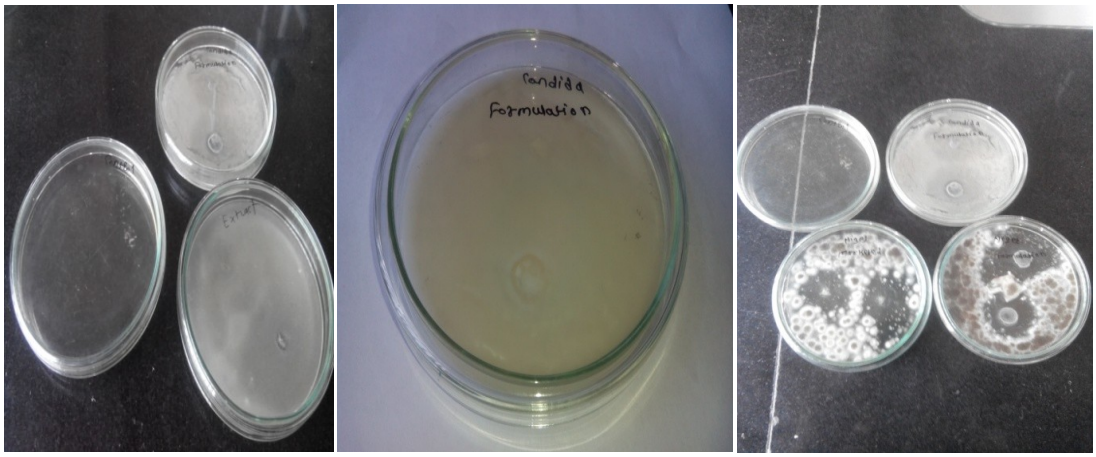


Fig. 4: Antifungal or Antipsoriasis Study of gel



5. Conclusion

Because of its having effective and easy to administer the topical formulation are widely accepted. Silymarin gel shows good viscosity with good antifungal activity. It was shown pseudoplastic flow property; good spreadability. Formulation stability shows stability up to two months at temp. 40^0C and no skin irritation in human volunteers.

Acknowledgment

The Authors are thankful to the trustees, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nasik for providing necessary research facilities.

Conflict of interest: None

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