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Original Article

Investigation on Potential of Karanjin Loaded Emulgel for Improved Efficacy against Psoriasis

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ABSTRACT

Aim: The objective of this work was to prepare and characterize Karanjin loaded emulgel for enhanced effectiveness against psoriasis. **Materials and Methods:** Karanjin emulsion was prepared using peppermint oil and was loaded in gel base (carbopol 940 was used as gelling agent). Various batches of emulsion were prepared by varying concentration of tween 80 and were optimized for various parameters. **Results:** Optimized emulsion (KE5) gave droplet size of 110.4 ± 1.56 nm, -40.9 ± 1.11 mV zeta potential, entrapment efficiency of 92.12 ± 2.8%, and creaming volume of 98.1 ± 0.4 and was found to be stable for 30 days during short term stability studies. The pH and viscosity of optimized Karanjin loaded emulgel was found to be 7.31 and 8060 cp respectively. *In-vitro* release of Karanjin from optimized emulgel was found to be 95.36% in 6 hr indicating slow release. *Ex vivo* permeation study in skin indicated 88% permeation of karanjin in 5 hr from optimized emulgel formulation compared to 16.52% permeation from solution formulation. *In vivo* study showed significant improvement in PASI score upon application of Karanjin loaded emulgel formulation. **Conclusion:** The results prove potential efficacy of the emulgel formulation in improving efficacy of Karanjin against psoriasis.
Keywords: Psoriasis, Emulgel, Karanjin, PASI Scoring, *In vivo* anti-psoriatic activity, *Ex vivo* permeation.

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INTRODUCTION

Psoriasis - a chronic autoimmune inflammatory condition of skin is characterized by abnormal growth of cells in the skin. This causes plaque formation on the skin, scaling following by itching, inflammation, redness and drying of the skin.[1] According to the WHO, about 125 million people have psoriasis, which accounts for 2-3% of the world population.[2] Although this disease is not life-threatening, it affects the quality of life of a patient badly. The therapy for psoriasis depends on the area of skin covered and the severity of disease. The systemic therapy and phototherapy are generally indicated in severe cases where, a high percentage of body surface area is covered. These treatments cause several adverse effects like liver toxicity and anti-proliferative action on healthy human tissues limiting use of the therapy for long-term.[3] The topical therapy for psoriasis is more preferred since it delivers the drug directly to the affected areas and is generally prescribed in mild to moderate cases.[4] Even topical anti-psoriatic medications exhibit several side effects like retinoids, corticosteroids, vitamin D analogs, anthralin and calcineurin inhibitors. They suffer from side effects like skin thinning, and resistance over a repeated application, skin irritation, phototoxicity and can be carcinogenic, too.[5] Therefore, there is need to develop alternative treatment with superior efficacy and lower side effects. Herbal candidates are beneficial because they have lesser side effects, improved therapeutic efficiency, and patient compliance.[6]

Pongamia pinnata - a flowering plant from the Fabaceae family, shows the anti-inflammatory effect and hence, it is used in treatment of psoriasis.[7] *Pongamia pinnata* is also known as Karanja, Malva nut and Karanj. All parts of Karanja are widely used in management of a wide range of wounds and diseases. Roots of this plant can treat gonorrhea, skin disease, ulcers, and vaginal disease. Seed oil is used for the treatment of scabies, ulcers and rheumatism. In addition, flavonoid derivatives including chalcones, flavones and flavans are major phytochemical excreted from the plant. Other compounds are also detected, such as sesquiterpenes, steroids, diterpene, triterpenes, amino acid derivatives. Karanjin is obtained from the seed, root, flower and stem bark of Karanja and chemically it is furoflavone.[8] Different formulations of Karanj are prepared, such as Karanj seed oil (devinez), Karanj capsule (psoravin capsule containing karanjchhal and neem leaves, bellan pharmaceutics), Karanj lotion (neemkarenjel lotion), Karanj ointment (imupsora).

The conventional topical formulation like creams, ointments, lotion suffers from problems like instability, stickiness, and less spreading coefficient.[9] Hence, a novel formulation – emulgel,

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which combines the advantages of both emulsion and gel was used to formulate Karanjin oil formulation. Emulsion helps in solubilization of hydrophobic drug molecules,[10] while gels offer faster drug release than other semisolid preparation,[11] and provide a higher spreading coefficient than an emulsion. Gel forms cross-linked networks in which small drug particles are captured and released in a controlled manner. It also exhibits mucoadhesive properties, which offers a prolonged contact time of formulation on the skin. Emulgel is a dual control release system that combine properties of both emulsion and gel. Gel for topical application offers unique advantages such as greaseless, easily spreadable, easily removed, emollient, and water-miscible. Moreover, incorporation of gel into an emulsion also result in improved emulsion stability and penetration ability by through its thixotropic behavior. Emulgel possesses several advantages, such as good patient acceptability; it contains non-greasy nature and does not require excess rubbing. Emulgel is a stable system and a better vehicle for hydrophobic drug molecules.[12]

The present work aims to investigate the emulgel potential for administration of Karanjin to skin in the management of psoriasis.

MATERIALS AND METHODS

Materials

Karanjin drug was provided by KV Naturals (Hyderabad, India). Peppermint oil, cinnamon oil, chamomile oil, coconut oil, and castor oil were procured from Avi Naturals (Hyderabad, India). Tea tree oil was procured from Vicciwin Pharma (India). Captex 300 and Captex 355 were procured from Gattefosse India Pvt. Ltd., Sorbitan monooleate (Span 80) and Sorbitan monolaurate (Span 20) were purchased from SD Fine Chemicals Pvt. Ltd., Polyethylene glycol 400 was obtained from LOBA Chemie. Carbopol 940 was purchased from Lubrizol. All additional chemicals and reagents were purchased commercially and were of analytical quality.

Solubility assessment of Karanjin in various oils

Screening of oils and emulsifiers was done by estimating the solubility of Karanjin in several oils. Briefly, an excess quantity of Karanjin was dispersed in 3 mL of various oils (peppermint oil, cinnamon oil, tea tree oil, castor oil, chamomile oil, coconut oil, captex 300, captex 355) in stoppered vials. The contents were uninterruptedly stirred for 72 hr using a vortex mixture to achieve equilibrium. Then, the contents were centrifuged for at 4000 rpm 15 min and the supernatant was filtered. The drug amount in resultant solution was analyzed by UV spectrophotometer (Shimadzu).[13]

Formulation of Karanjin loaded emulgel

To prepare Karanjin loaded emulsion, accurately weighed Karanjin was added to varying amounts of peppermint oil in a beaker and homogenized (1KA®T25, Ultra Turrex) for 10 min at 3000 rpm. The resultant oily phase was dispersed in an aqueous phase (tween 80 solution in water) using a homogenizer at 1000 rpm for 20 min.[14] The coarse emulsion, so formed was size reduced by high pressure homogenizer at 10 000 psi for 3 cycles. The formulation was kept in ice bath intermittently between the homogenization cycles to maintain temperature. Various batches of Karanjin-loaded emulsion were prepared by varying concentrations of emulsifier (Table 1).

Table 1: Composition of various batches of Karanjin loaded emulsion.

Ingredients	Composition						
	KE1	KE2	KE3	KE4	KE5	KE6	KE7
Karanjin (%w/v)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Peppermint oil (%w/v)	8	8	8	8	8	8	8
Tween 80 (%w/v)	0.5	0.75	1	1.25	1.5	1.75	2.00
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s	q.s

For the preparation of emulgel formulation, gelling agent-carbopol 940 was used.[15] Briefly, 1% carbopol 940 was dispersed on the optimized batch of emulsion and kept 24 hr at room temperature. Then, the contents were mixed through a 1KA-Eurostar stirrer at 100 rpm. 0.05% methylparaben was added to above gel and resultant was kept untouched for 12 hr to remove entrapped air.

Droplet size and zeta potential measurement

The droplet size of the prepared emulsion was estimated using Malvern Zetasizer ZS (Malvern instrument, U.K) by photon correlation spectroscopy.[16] Briefly, the various samples were suitably diluted with filtered distilled water and placed in a disposable cuvette. All measurements were performed in triplicate and the results are expressed as mean ± SD (standard deviation).

Percent entrapment efficiency and drug content measurement

For % entrapment efficiency, the emulsion was centrifuged at 5000 rpm for 15 min. The supernatant was collected and the drug amount was estimated in it by reverse phase high performance liquid chromatography (RP-HPLC). Unentrapped drug from formulation was removed by dialyzing the emulsion (filled in cellulose dialysis membrane) against double distilled water for 1 hr. Upon dialysis, the formulation was estimated and drug amount was calculated using the following formula,

$$\%\,EE = \frac{\text{amount of entrapped drug} \times 100}{\text{total amount of drug added}} \quad (1)$$

For determination of drug content, 50 mL of Karanjin loaded emulsion was dissolved in methanol and final volume was made up to 100 mL. The drug content in resultant solution was estimated using RP-HPLC method after appropriate dilution using methanol. For RP-HPLC method, Cosmosil® C_{18} column

(150 x 4.6 mm, 5 µm) was used and acetonitrile: 10mM KH_2PO_4 (50:50) was used as mobile phase. Detection was performed at 219 nm using UV detector. Sample injection volume was kept constant at 30 µL and flow rate was kept at 1.0 mL/min.[17]

Centrifugal stress study

1 mL of emulsion formulation was centrifuged for 15 min at 10,000 rpm. After centrifugation, the volume of lower phase was measured and the creaming volume (C) was calculated as follows:[18]

$$C = 100[v_t - v_s]v_t \quad (2)$$

Where, V_t is total volume of sample and V_s is volume of lower phase.

Short term stability study

All the batches were subjected to short term stability study at 25°C and 4°C. Various parameters (creaming, phase separation, coalescence and precipitation) were checked intermittently for 30 days. Zeta potential and droplet size were also measured.[19]

Characterization of Karanjin loaded emulgel

The formulation was evaluated for color, consistency, homogeneity and phase separation.[20] The pH of Karanjin loaded emulsion and the gel was checked by a pH meter.[21] The viscosity was measured by Brookfield viscometer,[22] (LVT model) using Helipath T-bar spindle at 12 rpm and 30°C ± 2°C. The spindle was added into the beaker containing formulation to measure viscosity.[23] The spreadability of emulgel was estimated using a glass slide as per the reported method,[24] and the spreadability coefficient was calculated. The drug content in emulgel formulation was also measured as per the process reported earlier for Karanjin emulsion.

In vitro release of Karanjin from Karanjin loaded emulgel formulation

In vitro release of Karanjin was carried out using Orchid Franz Diffusion cell. Cellophane membrane (12 kDa) of 2.5 cm^2 was soaked overnight in Phosphate buffer pH 7.4 and sealed between receptor and donor compartment. Formulation equivalent to 15 mg karanjin was evenly placed onto the dialysis membrane's surface at the donor compartment side. Phosphate buffer pH 7.4 (50 mL) was added in receptor compartment and stirred using a magnetic stirrer. Temperature and rpm were set at 34°C and 100 rpm, respectively. 5 mL aliquot samples were collected at fixed time intervals and the same amount of fresh media was replenished to the compartment. Amount of Karanjin in samples were estimated using RP-HPLC and cumulative karanjin release was calculated.[25] The study was performed for optimized Karanjin emulsion (KE5), Karanjin loaded emulgel formulation and karanjin solution (in methanol).

Ex-vivo permeation of Karanjin from Karanjin loaded formulation

Ex-vivo permeation of optimized Karanjin loaded emulsion (KE5), Karanjin loaded emulgel and Karanjin solution (in methanol) was investigated through rat skin. Briefly, euthanized rat skin was taken, rinsed with isotonic solution and was preserved in phosphate buffer-pH 7.4. The study was performed in modified franz-diffusion cell as described in literature with some modification.[20] Samples containing 15 mg of Karanjin were placed in donor compartment while 50 mL of 10% methanolic buffer (pH 7.4) was placed in receptor compartment. The temperature and rpm were set at 50 rpm and 37 ± 0.5°C respectively. An aliquot of 1 mL was withdrawn from receptor compartment at predetermined time points and media was replenished using fresh media. The samples were filtered using 0.45 µm syringe filter and Karanjin was estimated by developed method using RP-HPLC.

Assessment of anti-psoriatic activity

The subsequent anti-psoriatic activity assessment studies in animals were permitted by the institutional animal ethics committee, Ramanbhai Patel College of Pharmacy (Protocol: RPCP/IAEC/20-21/10). The experimental methods in the studies were in accordance to guidelines of the committee for control and supervision of experiments on animals, Government of India. For the study, BALB/c male mice were used for the study. The animals were divided as 3 groups of psoriatic animals (disease control, karanjin loaded emulgel and karanjin solution) and one group of non-psoriatic animal (normal control) with 6 animals in each group. The psoriasis – like lesions were induced on mice skin on dorsal side through topical administration of Imiquimod as per reported procedure with few modifications.[26] Cyclophosphamide at the dose of 3 mg/kg body weight was injected intraperitoneally to the mice on their dorsal side for 7 days. On 4th day, the hair on dorsal side of mice were removed and betadine solution was applied on it and left for 24 hr. 60 mg of Imiquad cream (each 0.25 gm cream contains 12.5 mg Imiquimod) equivalent to 3 mg of Imiquimod was applied over the skin surface of animals for 7 consecutive days (5-11th day of study period) except in normal control group.[27] Upon induction of psoriasis, anti-psoriatic activity of karanjin loaded emulgel was investigated and compared its efficacy with the activity of karanjin solution. Karanjin loaded emulgel and karanjin solution formulations were applied on the skin of animals for 5 consecutive days starting from 9th day of study period (5th day of Imiquimod application). The inflammation severity was assessed on each alternate day (on 4, 6, 8, 10 and 12th day of study period) on 0-4 scale of Psoriasis Area and Severity Index (PASI) scoring methodology. The scale for scoring was based on the skin thickness and erythema. The scale was referred as follows: 0- No effect, 1- Slight, 2- Moderate, 3-Severe, 4-Very severe.

Stability study

Optimized Karanjin loaded emulgel was subjected to stability studies. The formulation was stored in well closed glass container at room temperature for 90 days. The samples were analyzed at 0, 30, 60 and 90 days intervals. The parameters evaluated were consistency, % drug content, pH, phase separation and viscosity.

Statistical Analysis

All the data are expresses as mean ± SD (standard deviation). Data for various groups were subjected to statistical analysis by one-way ANOVA (analysis of variance) using GraphPad prism software (version 6.0, SanDiego, California). The value of *p* less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Assessment of solubility Karanjin in various oils

Selection of oil is an essential step in the preparation of emulsion since it influences the choice of other ingredients and is also necessary for preparing a formulation with high drug loading capacity; hence, the selection of oil was made quantitatively by calculating the maximum amount of drug soluble in 3 mL of various oils. The absorption maximum of Karanjin was obtained at 300 nm. The regression equation obtained was y=0.003x + 0.0024 with r = 0.9998 [calculated for for standard plot y-absorbance ratio to x-concentration in linear range of 2-10 µg/ml]. Figure 1 shows the graphical representation of the obtained results of solubility study of Karanjin. The drug's highest solubility was found in peppermint oil and hence, this oil was selected for further formulation development.

Formulation of Karanjin loaded emulgel

Various formulations of Karanjin emulsion were prepared as given in Table 1. The formulated emulsion batches were accessed for different parameters and the results obtained are shown in Table 2.

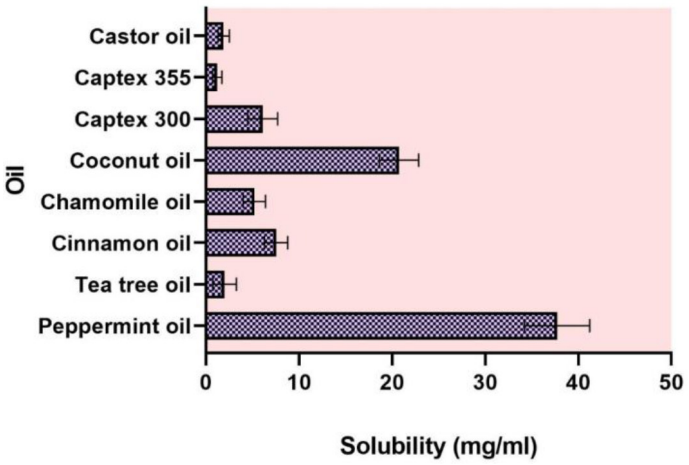

Figure 1: Result of solubility study of Karanjin in various oils.

Table 2: Results of evaluation parameters of various batches of Karanjin emulsion.

Batch no.	Droplet size (nm)	Polydispersity Index	Zeta potential (mV)	% Entrapment efficiency	Total drug content (mg)	Creaming volume
KE1	144.6 ± 1.18	0.2993 ± 0.03	-32.2 ± 0.26	86.2 ± 2.6	146.3 ± 1.82	82.3 ± 0.3
KE2	133.7 ± 2.22	0.256 ± 0.02	-36.2 ± 1.21	89.3 ± 2.1	144.4 ± 1.65	88.6 ± 0.2
KE3	126.5 ± 2.12	0.111 ± 0.02	-38.3 ± 0.63	89.6 ± 2.5	145.6 ± 1.81	94.1 ± 0.2
KE4	121.3 ± 1.13	0.162 ± 0.02	-40.6 ± 0.98	91.5 ± 3.2	144.8 ± 1.75	96.0 ± 0.2
KE5	110.4 ± 1.56	0.121 ± 0.02	-40.9 ± 1.11	92.12 ± 2.8	146.2 ± 2.10	98.1 ± 0.4
KE6	103.1 ± 1.66	0.112 ± 0.03	-43.3 ± 1.33	93.22 ± 2.8	144.2 ± 2.62	99.5 ± 0.4
KE7	96.4 ± 2.12	0.109 ± 0.02	-44.2 ± 1.42	95.28 ± 2.9	143.6 ± 2.82	99.3 ± 0.5

The result suggested that the droplet size was in 96.4 ± 2.12 to 144.6 ± 1.18 nm range and zeta potential in -32.2 ± 0.26 to -44.2 ± 1.42 mV range for all batches of emulsion formulation. It was found that as tween 80 concentration was increased, the droplet size and zeta potential gradually decreased. The zeta potential is an essential factor for consideration of the stability of biphasic systems like an emulsion. It indicates the repulsion force degree between two adjacent droplets in an emulsion.[28] Theoretically, high values of zeta potential on either side stabilize the biphasic system.[29] Hence, the value of zeta potential for all batches indicated good stability of the emulsion. The negative value of zeta potential could be due to anionic groups of fatty acids in peppermint oil and tween 80.

Percent entrapment efficiency, drug content and centrifugal stress study

The % EE was estimated by RP-HPLC method. The regression equation of karanjin was y = 1.982x + 0.0121 and r^2 was 0.998. LOD of drug was observed to be 0.01 µg/ml indicating that it is sensitive method of detection. Highest drug entrapment was obtained in batch KE7, while creaming values were higher for batches KE5 to KE7 (from 98-99%) as compared to other batches. The creaming values from centrifugal stress give a quick estimation of emulsion systems' stability.[30] Higher creaming values in batches KE5 to KE7 indicates good stability compared to other batches.

Short term stability study

Short term stability studies were conducted at 25°C and 4°C to check stability. The results are shown in Tables 3 and 4. Phase separation was observed in all batches upon storage at 25°C

Table 3: Droplet size values and emulsion batches.

Batch no.	Temperature (°C)	Droplet size (nm)					
		Day 0	Day 1	Day 5	Day 12	Day 20	Day 30
KE1	4°C	144.6 ± 1.18	150.2 ± 2.02	177.8 ± 2.81	202.3 ± 2.51	236.3 ± 2.63	261.1 ± 2.22
	25°C	144.6 ± 1.18	356.1 ± 2.22	Phase separation	-	-	-
KE2	4°C	133.7 ± 2.22	162.2 ± 2.02	170.0 ± 3.13	180.2 ± 2.01	212.3 ± 2.59	243.3 ± 2.12
	25°C	133.7 ± 2.22	311.8 ± 2.26	543.1 ± 2.33	Phase separation	-	-
KE3	4°C	126.5 ± 2.12	140.0 ± 2.32	161.8 ± 2.52	175.6 ± 1.89	203.1 ± 2.05	222.6 ± 2.31
	25°C	126.5 ± 2.12	251.3 ± 2.56	414.4 ± 2.32	602.3 ± 3.13	Phase separation	-
KE4	4°C	121.3 ± 1.13	136.3 ± 2.17	144.3 ± 2.62	170.3 ± 2.12	182.3 ± 3.10	203.7 ± 2.33
	25°C	121.3 ± 1.13	222.3 ± 2.16	313.1 ± 2.82	558.3 ± 3.11	Phase separation	-
KE5	4°C	110.4 ± 1.56	110.5 ± 1.12	111.3 ± 1.81	111.5 ± 1.20	112.7 ± 1.23	112.3 ± 1.72
	25°C	110.4 ± 1.56	120.6 ± 1.13	210.3 ± 2.12	323.2 ± 2.15	552.3 ± 3.12	Phase separation
KE6	4°C	103.1 ± 1.66	103.4 ± 1.24	104.7 ± 1.32	106.8 ± 1.62	106.2 ± 1.51	107.8 ± 1.89
	25°C	103.1 ± 1.66	121.1 ± 1.41	205.3 ± 2.72	320.1 ± 2.10	550.2 ± 3.10	Phase separation
KE7	4°C	96.4 ± 2.12	96.3 ± 1.72	97.1 ± 1.63	97.6 ± 1.42	98.3 ± 1.36	100.3 ± 2.36
	25°C	96.4 ± 2.12	110.2 ± 1.12	200.4 ± 2.71	302.3 ± 2.05	514.1 ± 3.33	Phase separation

*All the values are expressed as mean ± S.D (n=3).

Table 4: Zeta potential values of emulsion batches.

Batch no.	Temperature (°C)	Zeta potential (mV)					
		Day 0	Day 1	Day 5	Day 12	Day 20	Day 30
KE1	4°C	-32.2 ± 0.26	-35.2 ± 1.12	-37.6 ± 1.52	-39.2 ± 1.22	-42.3 ± 1.27	-45.6 ± 2.15
	25°C	-32.2 ± 0.26	-40.2 ± 1.11	-	-	-	-
KE2	4°C	-36.2 ± 1.21	-38.9 ± 1.37	-40.0 ± 1.16	-41.1 ± 1.27	-42.6 ± 1.38	-44.3 ± 2.16
	25°C	-36.2 ± 1.21	-40.2 ± 1.23	-43.3 ± 1.62	-	-	-
KE3	4°C	-38.3 ± 0.63	-40.2 ± 2.36	-41.6 ± 1.37	-42.5 ± 1.29	-44.6 ± 1.30	-47.6 ± 1.18
	25°C	-38.3 ± 0.63	-41.7 ± 1.26	-42.3 ± 1.37	-44.5 ± 1.15	-	-
KE4	4°C	-40.6 ± 0.98	-41.2 ± 1.12	-43.8 ± 1.73	-45.6 ± 2.17	-45.2 ± 1.19	-47.5 ± 1.29
	25°C	-40.6 ± 0.98	-42.1 ± 2.36	-44.6 ± 1.20	-47.5 ± 1.10	-	-
KE5	4°C	-41.1 ± 1.11	-41.2 ± 1.12	-40.6 ± 1.46	-41.7 ± 1.86	-41.3 ± 1.20	-42.5 ± 1.26
	25°C	-41.1 ± 1.11	-44.3 ± 1.26	-44.8 ± 1.28	-48.2 ± 1.30	-50.3 ± 1.18	-
KE6	4°C	-43.3 ± 1.33	-43.2 ± 1.16	-43.6 ± 1.27	-43.8 ± 1.38	-43.5 ± 1.15	-43.8 ± 1.17
	25°C	-43.3 ± 1.33	-45.2 ± 2.46	-45.2 ± 1.22	-47.3 ± 2.18	-51.5 ± 2.15	-
KE7	4°C	-44.2 ± 1.42	-44.3 ± 1.17	-44.8 ± 1.22	-44.2 ± 1.40	-43.6 ± 1.18	-45.6 ± 1.10
	25°C	-44.2 ± 1.42	-46.3 ± 1.31	-47.5 ± 1.52	-48.2 ± 1.33	-51.3 ± 1.36	-

*All the values are expressed as mean ± S.D (n=3).

within 30 days, signifying their instability. While at 4°C, the phases did not separate. There was a significant increase in droplet size (for samples kept at 4°C) in batches KE1 to KE4, probably because of an insufficient concentration of tween 80 to stabilize the biphasic system. Batches KE1 to KE4 showed significant decrease in zeta values, while there was no significant change for batches KE5 to KE7 (P> 0.05). The stability study suggested that batches KE5, KE6, and KE7 were stable for one month at 4°C. Thus, KE5 (having lower surfactant concentration) was selected as an optimized emulsion batch and was taken for emulgel formulation.

Characterization of emulgel

Formulated emulgel of Karanjin was obtained as creamy white formulation with a smooth, even consistency and slightly glossy appearance. Phase separation was not observed. The pH and viscosity of emulgel formulation were found to be 7.31 and 15650 cp, respectively. Good spreadability is an essential criterion for an emulgel formulation. It depends on parameters like the viscosity of preparation and the physical properties of polymers in the preparation. Generally, a highly viscous formulation shows poor spreadability, and it directly affects the therapeutic efficacy of the preparation. In this study spreadability of the formulation was

estimated in terms of spreading coefficient and was estimated to be 11.7 g cm/sec. The positive value obtained for the spreading coefficient indicates that the formulation possesses good spreading property.

In vitro release of Karanjin from Karanjin loaded emulgel formulation

The *in vitro* release profiles of Karanjin from the optimized emulsion and emulgel formulation are shown in Figure 2. About 95% of Karanjin was released in 1 hr from karanjin solution while Karanjin emulsion and gel formulation gave a cumulative drug release of 94.32 ± 11.18% in 3 hr and 95.36 ± 9.27% in 6 hr respectively. The slow drug release from emulgel formulation could be due to creating a viscous environment surrounding the drug molecules.[31] As these formulations were able to slow down release of Karanjin, they could be considered suitable for enhanced effectiveness against psoriasis since Karanjin got available for longer time locally on the skin.

Ex vivo permeation of Karanjin from Karanjin loaded formulation

Ex vivo permeation was performed to reconnoiter the behavior of transport of Karanjin through the skin. The results obtained are shown in Figure 3. Karanjin solution showed only 16.52 ± 2.65% drug permeation within an hour. This data shows that although drug release from solution was 96% (as obtained in *in vitro* release study), the permeation of Karanjin was observed to be poor. Moreover, the permeation rate was observed to be slower in case of emulgel formulation (88% in 5 hr) which signifies its utility for topical application. Faster rate in case of emulsion formulation (87% in 3 hr) may be undesirable since Karanjin may not be retained for longer duration of time on the skin.[32]

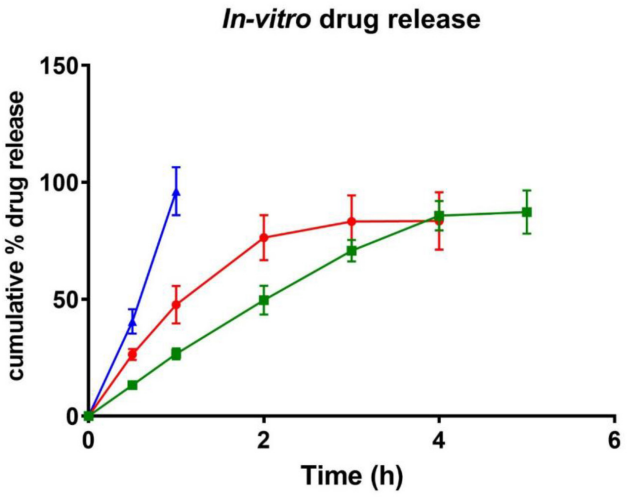

Figure 2: *In vitro* release of Karanjin from Karanjin solution (-▲-), optimized Karanjin loaded emulsion (-●-) and Karanjin loaded emulgel (-■-).

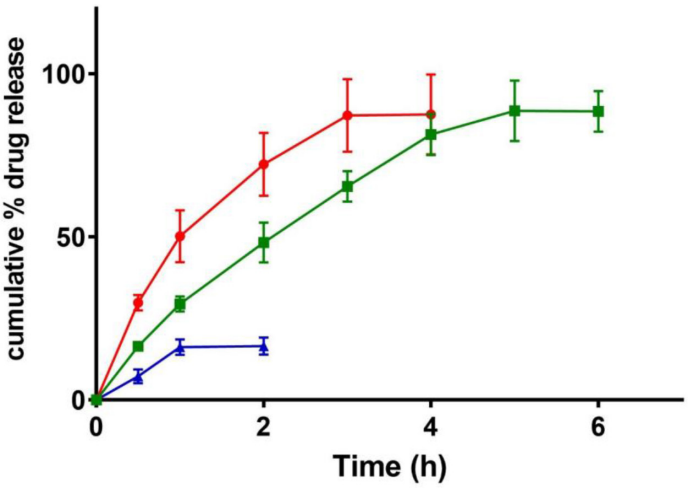

Figure 3: *Ex-vivo* permeation of Karanjin from solution formulation (-▲-), optimized Karanjin loaded emulsion (-●-) and Karanjin loaded emulgel (-■-).

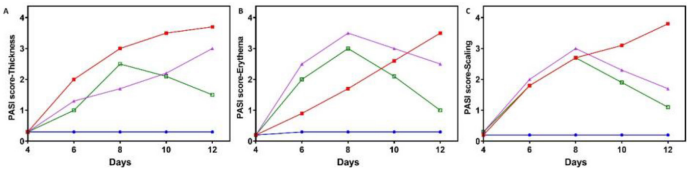

Figure 4: Graphical representation of PASI scores of (a) Skin thickness, (b) erythema and (c) scaling for four groups of animals: Normal Control (-●-), Disease Control (-■-), Karanjin solution (-▲-) and Karanjin loaded emulgel (-□-).

Assessment of anti-psoriatic activity

The efficiency of formulation to decrease psoriasis was investigated by Imiquimod induced psoriasis model in mice. Psoriasis was scaled (0-4 scale) in terms of thickness of skin, erythema and scaling on skin. The results are depicted in Figure 4 and comparative graph of morphology of skin of animals in 4 groups are depicted in Figure 5. The scoring for all three parameters were set to zero for normal control group. In case of disease control, there was increase in PASI scores indicating increased thickness, erythema and scaling. In case of group who received solution formulation, the PASI scores increased at all days but scoring was significantly less ($p< 0.01$) as compared to disease control. For group which received karanjin loaded gel formulation, PASI score decreased significantly ($p<0.01$) as compared to disease control and solution formulation. This could be due to increased permeability of Karanjin from gel formulation.

Stability study

Table 5 shows the results of stability study. Consistency, pH and viscosity of optimized Karanjin loaded emulgel was found to be consistent over the study period. No signs of phase separation was observed. Moreover, Karanjin content was found to be stable with non-significant difference over the study period ($P<0.01$)

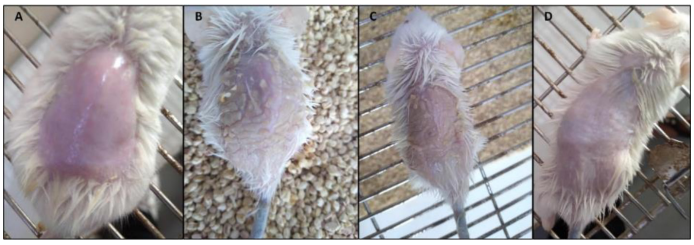

Figure 5: Comparative graph of skin morphology of an animal from each group: (a) Normal Control, (b) Disease control, (c) Karanjin loaded solution and (d) Karanjin loaded emulgel formulation.

Table 5: Result of stability study of optimized Karanjin loaded emulgel over 90 days period.

Evaluation parameter	Day			
	Day 0	Day 30	Day 60	Day 90
Consistency	Acceptable	Acceptable	Acceptable	Acceptable
% Drug content	97.23 ± 0.17	97.21 ±0.18	97.26 ± 0.11	97.18 ± 0.16
pH	7.31 ± 0.15	7.31 ± 0.18	7.30 ± 0.17	7.30 ± 0.16
Phase separation	None	None	None	None
Viscosity (cP)	15650 ± 31	15656 ± 25	15641 ±30	15645 ± 26

*All the values are expressed as mean ± S.D (*n*=3).

indicating no deterioration of drug during storage at room temperature.

CONCLUSION

Karanjin loaded emulgel was formulated and various parameters were estimated. The optimized emulgel batch having desired values of droplet size was evaluated for several parameters such as pH, viscosity and phase separation. Karanjin emulgel was stable for 30 days stability test. The formulation was then evaluated for anti-psoriatic activity in mice. From the result it was observed that Karanjin loaded emulgel was able to treat the psoriasis. Hence, it concluded that Karanjin loaded emulgel will offer a solution for topical application of Karanjin.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Span 80: Sorbitan monooleate (Span 80); **Span 20:** Sorbitan monolaurate (Span 20); **RP-HPLC:** Reverse phase high performance liquid chromatography.

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