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Development and optimization of *Cuscuta reflexa*-based hydrogel formulation for management of eczema: in vitro and in vivo approach

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Abstract

Background Eczema remains a challenging dermatological condition affecting millions worldwide and current treatments often present limitations and side effects. Dodder or *Cuscuta reflexa* has documented anti-inflammatory, antioxidant, and antimicrobial properties that may benefit skin conditions. The goal for this study was to develop and optimize a novel topical hydrogel with anti-eczema activity using *C. reflexa* extract for eczema management. Evaluation parameters included physicochemical characterization like viscosity, pH, spreadability, in vitro drug release, ex vivo permeation, skin irritation, and in vivo efficacy using DNCB-induced eczema model.

Results The hydrogel was formulated using varying concentrations of Carbopol 934 as the gelling agent. Response surface methodology optimized the formulation, yielding ideal physicochemical properties: viscosity (14,872 cP), pH (6.32), and spreadability 76%. In vitro studies demonstrated sustained release with 64.00% cumulative release over 14 h. Ex vivo permeation study optimized formulation showed 9% permeation and 48% retention in 4 h. Skin irritation study indicated that the optimized hydrogel formulation is safe for topical application without causing skin irritation. In vivo evaluation in an eczema model induced by 2,4-dinitrochlorobenzene (DNCB) in ICR mice showed significant improvement in clinical symptoms and histopathological parameters.

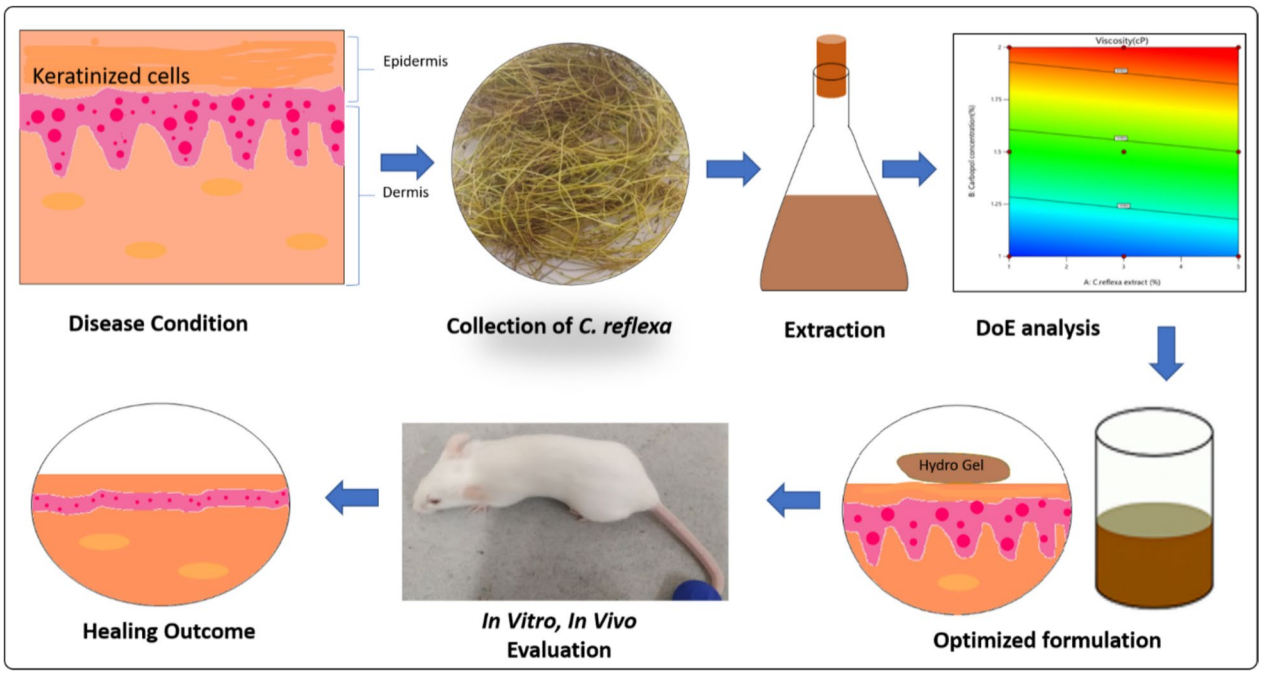
Conclusion The developed *C. reflexa* hydrogel formulation represents a promising natural alternative for eczema management, demonstrating optimized formulation, exhibits favorable physicochemical properties, sustained release characteristics, and therapeutic efficacy in eczema models.

Keywords *Cuscuta reflexa*, Eczema, Hydrogel, Topical application, Anti-inflammatory, In vitro drug release, In vivo efficacy

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Graphical Abstract



Introduction

Eczema, commonly recognized as atopic dermatitis, is a chronic inflammatory skin disease with severe pruritus. It affects around 15–20% of children and 1–3% of the adults in the global population [1]. Acute eczema is characterized by itching, erythema, edema, and vesiculation, whereas the clinical presentation of chronic eczema is thickening of the skin, lichenification, and scaling [2]. Presently available treatment modalities include topical corticosteroids, calcineurin inhibitors, emollients, and antihistamines, but these traditional topical agents are not well-tolerated for long-term use, especially corticosteroids, which are associated with several undesirable side effects, including atrophic changes of the skin, telangiectasia, and tachyphylaxis [3].

The pathophysiology of eczema is characterized by interactions between genetic predisposition, environmental exposures, skin barrier disruption, and immune dysregulation [4]. Abnormal skin barrier function results in trans epidermal water loss also through mediating the entry of allergens into the skin and innate immune response activation mediated by *T* helper (Th2) cells [5], whereas the skin microbiome is central; *Staphylococcus aureus* colonization may worsen the inflammation through the production of super antigen [6].

Due to the limitations of conventional therapies, interest in natural products with anti-inflammatory, antimicrobial, and barrier-restoring properties has been increasing. A new emerging field called "Phyto therapy" is providing safer treatment approaches with lesser side effect and attracting demand [7]. *Cuscuta reflexa* (family Convolvulaceae), also referred to as dodder or "Amarbel" has been used to treat a variety of skin disorders among other medicinal plants in traditional medicine due to its therapeutic potential [8].

Cuscuta reflexa is a widely distributed parasitic plant in tropical and temperate regions, and is prevalent in India, China, and other areas of Asia [9]. Ethnopharmacological literature of Cuscuta species articulates the various ancients' medicinal practices treating skin disorders due to the anti-inflammatory, antimicrobial, antioxidant, and wound-healing activities [10]. The phytochemical analysis shows the presence of many bioactive compounds like flavonoids (apigenin, quercetin, kaempferol), glycosides (cuscutin, amarbelin), coumarins, polyphenols, etc., which confer medicinal importance of this plant [11].

Recently, some of these claims have been evaluated in pharmacological studies. For example, *C. reflexa* extract was shown to have powerful anti-inflammatory activity by suppressing pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and blocking the NF-κB signaling

pathway [12]. It is also potently antioxidant and helps protect against oxidant harm, a key contributor in the pathogenesis of inflammatory skin conditions like eczema [13]. In addition, antimicrobial testing has shown activity against common skin pathogens, including *S. aureus*, which frequently colonizes eczematous skin [14].

Topical drug delivery systems have many benefits in the treatment of skin diseases including localized drug delivery, bypassing hepatic first pass metabolism, and better patient compliance [15]. Topical formulations containing hydrogels are well suited to the dermatological application as they are high in water, cooling, spreadable, and non-greasy among others [16]. In addition, hydrogels create a moist atmosphere that facilitates recovery of the wound and facilitate drug permeation through the stratum corneum [17].

Although the therapeutic potential of *C.reflexa* has been documented, and hydrogel is advantageous for drug delivery, there have been no early investigations on optimized and best *C. reflexa*-based hydrogel formulations that could be tailored for the management of eczema. Accordingly, the current study is planned to fabricate and optimize a new extract of *C. reflexa*-loaded hydrogel formulation and to evaluate its potential in in vitro and in vivo studies for eczema management.

Materials and methods

Materials

Cuscuta reflexa whole plant was collected from the botanical garden of Directorate of Medicinal and Aromatic Plants Research, Anand and authenticated by botanist Dr. B.B. Maitreya from the Department of Botany, Gujarat University. Carbopol 934 was purchased from Yarro chem, Mumbai, India. Hydroxypropyl methylcellulose (HPMC K4M), propylene glycol, methylparaben, propylparaben, and triethanolamine were procured from Sigma-Aldrich (USA), while all other reagents and chemicals used in the work were of analytical grade.

Experimental animal

The study was based on female Sprague–Dawley rats and ICR mice with a weight of 120–150 g and 20–35 g, respectively. Prior to initiating the study, approval was obtained from the Institutional Animal Ethics Committee (IAEC) as well as the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under registration number-883/PO/ReBi/S/05/CPCSEA.

Methods

Extraction of plant material

For extraction, the maceration technique was utilized, wherein 100 g of powdered *C. reflexa* was introduced into a conical flask containing 250 mL of ethanol and 250 mL of water. The flask was allowed to stand for a duration of 72 h, during which periodic stirring of the contents was conducted, subsequently followed by straining the contents of the extraction flask first through muslin cloth and afterward from a Whatman filter paper. Thereafter, the resultant was concentrated at 40 °C under reduced pressure until a dry residue was obtained. The yield (%) and physical characteristics of the extract were documented, and the final extract was stored at 4 °C in a refrigerator for later use [18, 19].

Phytochemical analysis

A preliminary investigation was conducted on various extracts using conventional methods in order to detect key phytoconstituents. The qualitative screening involved some confirmatory tests for the different classes of compounds, for example, for alkaloids, Mayer's, and Dragendorff's reagents were employed, flavonoids were detected through the Shinoda reaction, and phenols were detected via the ferric chloride test. Gelatin precipitation technique was used for tannin detection, while saponins were detected through foaming. For the presence of steroids and triterpenoids, the Liebermann–Burchard test was used, glycosides were established through Keller–Killiani technique, and carbohydrates were detected using Molisch's reagent [20].

Estimation of total phenolic and flavonoid content

The concentration of total phenolic compounds in the sample was determined using a modified Folin–Ciocalteu colorimetric method. In the technique, 0.5 ml of the extract (1 mg/ml) was added to 2.5 ml of 10% Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The reaction mixture was incubated at room temperature and allowed to stand for 30 min. A UV–Visible spectrophotometer (Shimadzu UV–1800, Japan) was used to measure the absorbance at 765 nm. Total phenolic content (TPC) was calculated as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) [21].

For determination of total flavonoid content (TFC), the aluminum chloride method was used. In a test tube, 0.5 ml of the extract (1 mg/ml) was added to 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. After 30 min of incubation at room temperature, the absorbance of samples was measured at 415 nm. TFC was calculated as mg quercetin equivalents per gram of extract (mg QE/g) [22].

Determination and quantification of apigenin by UV spectrophotometry

5 mg of the apigenin was taken and dissolved in 5 mL of a mixture of methanol, and the dried extract was dissolved in water to obtain a concentration of 1000 µg/mL to make a stock solution. This was used to prepare five additional standard solutions which were 2, 4, 6, 8, and 10 µg/mL with respect to the primary solution in order to establish a standard curve [23].

Development of C. reflexa extract-loaded hydrogel

To prepare the hydrogel, 1 g of Carbopol 934 was added slowly to 50 mL of distilled water and left overnight to hydrate. The dispersion was stirred to produce a homogeneous gel base. Methylparaben and propylparaben were dissolved in 5 mL of distilled water, and then added to the formulation. The solution was prepared under the heating on a water bath, and propylene glycol was added to the cooled solution. *Cuscuta reflexa* hydroalcoholic extract was used as a source of phytochemicals and added into the mixture. The rest of the formulation was made up to 100 mL with distilled water and uniformly mixed. TEA was added dropwise to obtain a pH between 6.8 and 7 magnitude that is skin-friendly and gave proper gel consistency. A control formulation was prepared identically but without the plant extract. The finished product was stored in collapsible polyethylene tubes under cool, dry conditions, and its physical properties including color, texture, etc., were noted [24]. The formulation details of the initial *C. reflexa*-based hydrogel are summarized in Table 1.

Optimization of C. reflexa extract-loaded hydrogel by factorial design

Based on the preliminary findings, a full 3^2 factorial design was employed. In this design, two formulation variables were analyzed independently at three concentration levels, amounting to a total of nine unique combinations. Two separate variables were used in three level factorial trials. The *C. reflexa* extract (*X*1) and Carbopol concentration (%) (*X*2) were selected as free variables, and design included two main responses: %CDR (*Y*1) and Viscosity (*Y*2) [25, 26]. The experimental design matrix is presented in Table 2.

Characterization of hydrogel formulations

Visual inspection of formulations

The formulated hydrogel samples were subjected to grading of color, uniformity, texture, and the presence of clumping, phase separation, or other inconsistencies.

Determination of pH

For pH analysis, 1 g of hydrogel was dispersed in 10 mL of distilled water and allowed to stand for 5 min. The sample was then subjected to temperature control at 25 ± 2 °C and measured using a Mettler Toledo pH meter after electrode immersion of the swollen hydrogel mixture [27].

Spreadability test

The assessment of hydrogel spreadability was achieved through a slide method. 0.5 g of the gel was placed between two glass slides. Subsequently, a 100-g weight was added onto the upper slide for 5 min to eliminate trapped air and yield a consistent gel layer. The initial weight was removed and substituted with a 20-g mass. The time needed to slide the top the slide a distance of

Table 2 Factors and their levels as per 3^2 factorial design for optimization of C. reflexa hydrogel formulation

Independent formulation variables			
Independent variables	**Low (− 1)**	**Medium (0)**	**High (+ 1)**
Cuscuta reflexa extract (%) (*X*1)	1%	3%	5%
Carbopol concentration (%) (*X*2)	1%	1.5%	2%
Dependent variables: *Y*1 = %CDR and *Y*2 = VISCOSITY			

Table 1 Composition of *C. reflexa* extract-based hydrogel formulations (CRE1–CRE5)

Ingredients	CRE 1	CRE 2	CRE 3	CRE 4	CRE 5
Carbopol 934	1%	1.5%	2%	1.5%	1.5%
Cuscuta extract	1%	1%	1%	3%	5%
Propylene glycol	5%	5%	5%	5%	5%
Methyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%
Propyl paraben	0.1%	0.1%	0.1%	0.1%	0.1%
Triethanolamine	q.s	q.s	q.s	q.s	q.s
Distilled water	up to 100 g	up to 100 g	up to 100 g	up to 100 g	up to 100 g

q.s. = Quantum satis (quantity sufficient to achieve gel consistency and adjust pH); distilled water was added to complete the total weight of the formulation up to 100 g

10 cm was noted. Spreadability (S) is computed using the following formula:

$$S = m * l/t$$

where m is the weight (g) applied, l is distance moved (cm), and t is time in seconds [28].

Determination of viscosity

Measurement of viscosity was performed on a Brookfield viscometer (Model DV-II + Pro, USA) equipped with spindle 64 at 20 rpm. All measurements were conducted at room temperature (25 ± 2 °C) [29].

Determination of drug content

For quantification of the drug incorporated into the hydrogel, a sample of 1 g was dissolved in 100 mL of phosphate buffer, pH 6.8, followed by sonicating the solution for 30 min. Subsequently, the solution was filtered through a 0.45-µm membrane and UV spectrophotometry was used to determine the maximum amplitude absorption wavelength (λmax). A pre-set calibration curve was employed for finding the content of the drug [29].

Determination of extrudability

The extrudability of hydrogels was evaluated using a collapsible polyethylene tube filled with 20 g of hydrogel. The tube was compressed at the sealed end, and the extruded length was measured when a weight of 1 kg was applied. The extrudability was expressed as the extruded length (cm) of hydrogel from the tube [30].

In vitro drug release studies for optimized hydrogel formulation

Cuscuta reflexa extract was determined to be released from the manufactured hydrogel by use of a modified Franz diffusion cell with a membrane serving as the diffusion barrier. In the study of the drug permeation, we set up a dialysis membrane between the donor and receptor compartments in a modified Franz diffusion apparatus and stored in saline phosphate buffer (pH 7.4) for an entire day before to the investigation. One gram of hydrogel was put on the dialysis membrane. The receptor compartment was filled with 17 mL of phosphate-buffered saline, PBS (pH 7.4) as a dissolution medium maintained at 37 °C to mimic physiological conditions, continuously agitated at 150 rpm to provide homogeneity. The donor and receptor chambers remained in constant contact with one another during the experiment. To ensure sink conditions, 1 mL aliquots were removed from the medium on the receptors at predetermined time intervals of 2, 4, 6, 8, 10, 12 and 14 h and an equal amount of PBS immediately replaced. The withdrawn samples were examined spectrophotometrically at 274 nm to determine the cumulative drug release profile of the formulation. The in vitro drug release study of the prepared hydrogel was also compared with a marketed herbal cream formulation. The dissolution parameter T50 (time required for 50% drug release), was calculated by linear interpolation between the two consecutive sampling points that enclosed the 50% release value [31].

Ex vivo skin permeation studies

In this investigation of ex vivo permeation, freshly removed Sprague–Dawley rat skin was used as a substitute for human skin. Subcutaneous fat was carefully removed so as not to cause skin damage. Before being used, the skin was defrosted at room temperature after being kept at − 20 °C before use. The skin samples were placed between the donor and receptor sections of a Franz diffusion cell for the permeation studies, after being pre-hydrated for 30 min in phosphate-buffered saline (PBS). To replicate physiological skin conditions, PBS was added to the receptor compartment and kept at a temperature of 32 °C. The sample compartment was continuously stirred using a magnet. The donor chamber's skin surface received a precise dosage of the medication formulation before it was sealed to stop evaporation. In order to preserve sink conditions, receptor fluid samples were withdrawn at certain time intervals of 2, 4, 6, 8, 10, 12, and 14 h, samples were taken and replaced with fresh PBS to keep sink conditions. After completion of the permeation study, the skin samples were carefully excised and residual formulation adhering to the surface was gently removed. Various layers of the skin were removed in sequence by the tape-stripping method, the formulation was extracted from the strips, and the amount of drug retained in the skin was calculated using the equation:

$$\text{Drug retention } (\mu g/g \text{ tissue}) = \frac{\text{Concentration determined} * \text{Volume of extraction}}{\text{Weight of sample}}$$

The samples collected were examined with UV–Visible spectrophotometry at 274 nm, the maximum absorption wavelength of the drug. The ex vivo permeation results of the prepared hydrogel were also compared with those of a marketed herbal cream formulation [32].

Animal study

Skin irritation studies The potential for dermal irritation was assessed using an adapted Draize protocol. Healthy female Sprague–Dawley rats weighing between 150 and 200 g were split into three different groups of six per group:

Group I: Untreated control.

Group II: Irritated using 0.8% v/v formalin as a standard irritant treatment.

Group III: Optimized hydrogel formulation.

through which the agents for testing were administered. The rats' back was shaved 24 h before the experiment. The substances to be tested were applied to the shaved part of the body, and then, the area was covered with the adhesive tape. After 72 h of the experiment, the tape was pulled off, and the skin was evaluated using the Draize scoring system for erythema and edema (0 = No change observed, 1 = Slight response, 2 = Moderate response, 3 = severe reaction, 4 = Very severe reaction). The Primary Irritation Index (PII) was calculated as the mean of the total scores for erythema and edema versus the number of animals evaluated [33].

In vivo efficacy studies in DNCB-induced eczema model The in vivo efficacy of the optimized hydrogel formulation was evaluated using 2,4-dinitrochlorobenzene (DNCB)-induced eczema model in ICR mice. Healthy female ICR mice (30–45 g) were divided into five experimental sets ($N = 6$ for each set): Group I: Normal control with no treatment, Group II: Negative control with dermatitis induced by DNCB, Group III: Standard, Marketed formulation, Group IV: placebo hydrogel without extract, and Group V: optimized *C. reflexa* hydrogel.

For the initiation of sensitization, 100 µl of 0.5% DNCB added into a solution of acetone and olive oil in 2:1 ratio was applied on the ears of mice for three consecutive days. After sensitization, the animals were challenged with 20 µl of 1% DNCB two times a week for two weeks to induce eczema-like skin lesions. The test substances were applied topically to the lesions twice daily starting from the challenge phase [34].

Evaluation of eczema severity The intensity of the dermatitis symptoms were assessed during the induction and the treatment of the eczema model on 4, 7, 14, and 28 days. A scoring system was applied to the examination of the damaged skin regarding its redness (erythema), thickening (lichenification) and skin-scratching (excoriation), flaking (scaling), and swelling (edema). Symptoms were scored between 0 and 4: 0 for no symptoms and 4 for severe symptoms. The overall skin condition was quantified by adding the scores of all four parameters, producing a total dermatitis severity score [31].

On the day after the last model construction and treatment (Day 29), the treatment effects were assessed by conducting measurements. The thickness of the treated ear was measured with a digital thickness gauge (Model 547-500S, Mitutoyo Corp., Japan), and the body weights were noted down. After this, the mice were sacrificed so that ear skin and spleen samples could be gathered. The spleen was weighed, and the spleen index, which is the ratio of the spleen weight to the body weight, was calculated. Subsequently, the collected ear skin was subjected to histological examination [35].

Stability studies The hydrogel formulation development was completed alongside stability testing in accordance with ICH Q1A(*R*2) criteria. Samples were kept under several conditions: 5 ± 3 °C (refrigerated), 25 ± 2 °C with 60 ± 5% relative humidity, and 40 ± 2 °C with 75 ± 5% RH over a period of three months. As set in the timeline, (1, 2, and 3 months), the formulations were examined in triplicate for their appearance, pH, drug content, viscosity, and in vitro permeation efficacy [36].

Statistical analysis Results are shown in the form of mean values with standard deviation (SD), which is calculated from at least three independent trials. For statistical evaluation of significance, one-way ANOVA was used followed by Tukey's multiple comparison test between the groups in GraphPad Prism software version 8.0; GraphPad Software Inc., USA). A p-value threshold of 0.05 was established as statistically significant.

Results

Extract yield

The maceration process with water and ethanol effectively extracted the plant matter effectively to give a 21% yield of a dark brown extract.

Phytochemical analysis

The hydro alcoholic extracts showed the presence of several phytoconstituents (Table 3). Notable key phytochemicals of primary significance like alkaloids, flavonoids, phenolic compounds, saponins, tannins,

Table 3 Phytochemical screening of *C. reflexa* extract

Sr. No	Phytochemical group	Detection in hydroalcoholic extract
1	Alkaloid compounds	–
2	Flavonoid compounds	+
3	Phenolic derivatives	+
4	Tannin compounds	+
5	Saponins	–
6	Steroidal/triterpenoid	+
7	Glycosides	+
8	Carbohydrates	+

Key: "+" indicates presence; "–" indicates absence

Table 4 Total amounts of flavonoids and phenolic compounds in extracts of *C. reflexa*

Sr no	Chemical class	Content (mg/g dry material)	Standard used
1	Flavonoid	84.50	Quercetin (QCT)
2	Phenolic	35.56	Gallic acid (GAE)

Table 5 Characterization of preliminary *C. reflexa* extract-loaded hydrogel formulations

Formulation code	Viscosity (cP)	pH	%CDR
CRE-01	5000	6.4	66.00
CRE-02	15,000	6.5	60.00
CRE-03	20,000	6.3	56.00
CRE-04	16,000	7.1	65.00
CRE-05	17,000	7.2	66.00

glycosides, and a significant amount of carbohydrates were revealed during qualitative screening of hydroalcoholic extracts.

Estimation of total phenolic and flavonoid content

The total amount of flavonoids and phenolic compounds in the plant extract were assessed by the colorimetric method measuring at 510 nm with quercetin as a standard and the Folin–Ciocalteu method at 750 nm using gallic acid, respectively. The total flavonoid content of the extract was determined to be 84.50 mg/g (quercetin equivalent), while the total phenolic content was 35.56 mg/g (gallic acid equivalent) (Table 4).

Determination and quantification of apigenin by UV spectrophotometry

The apigenin content in the extract, quantified by UV–Visible spectrophotometry at 268 nm, was found to be 7.69%.

Development and characterization of preliminary hydrogel formulations

Five preliminary hydrogel formulations (CRE1-CRE5) were prepared using different concentrations of Carbopol 934 (1–2% w/w). All formulations were transparent and uniform, with no phase separation or aggregation. The pH of the formulations ranged between 6.3 and 7.2, viscosity between 5000 and 17000 cP, and % cumulative drug release (%CDR) at 14 h ranged from 56 to 66% (Table 5).

Table 6 Experimental design matrix and observed responses for *C. reflexa* hydrogel formulations

Formulation Code	X_1 (% w/w)	X_2 (% w/w)	%CDR	Viscosity (cP)
CRE-01	1	1	66	5000
CRE-02	1	1.5	60	15,000
CRE-03	1	2	56	20,000
CRE-04	3	1	66	5500
CRE-05	3	1.5	65	16,000
CRE-06	3	2	63	21,000
CRE-07	5	1	69	6000
CRE-08	5	1.5	66	17,000
CRE-09	5	2	61	22,000

Table 7 Summary of regression analysis findings for responses *Y*1 and *Y*2

Response	Std. Dev	R^2	Adjusted R^2	Predicted R^2	C.V. %
*Y*1	1.83	0.8411	0.7881	0.6362	2.88
*Y*2	1605.11	0.9593	0.9458	0.9151	11.33

Optimization of hydrogel formulation using a 3^2 factorial design

A 3^2 factorial design was used to guide the *C. reflexa*-based hydrogel formulation design. Selected independent variable pairs include: X1: Concentration of Extract of *C. reflexa*. *X*2: % Carbopol 934 concentration. Preliminary observations suggest measuring these responses: *Y*1: % CDR and *Y*2: Viscosity of the formulation. The experimental design matrix and observed responses (Table 6). Experimental combinations alongside measured outcomes, namely, %CDR and viscosity, ranged from 56 to 66% and 5000–20,000 cP, respectively. The collected responses were subsequently analyzed statistically utilizing response surface.

The obtained results were then statistically analyzed using response surface methodology and ANOVA. The effects of independent variables on dependent response were further analyzed by response surface plot and the related polynomial model. The data were evaluated by Design Expert software. A linear model was found to be the best about the fitting of %CDR and viscosity. The results fit statistics are shown in Table 7, primary indicators were proved effective in regression models and those being adjusted and predicted R^2, secondary adjusted standard deviation model fitness capability, R^2 coefficient best predicting the outcome and variation (%CV) proved capable.

Effect of independent factors on %CDR (*Y1*)-surface response study

For the nine trials, %CDR values fitted within the range of 56–69%.

$$Y1 = +70.56 + 1.17 * X1 - 7.00 * X2 \quad (1)$$

As per polynomial Eq. (1) and counter plot (Fig. 1A), in this model, the constant (70.56) is the theoretical %CDR when the two independent variables are at their central values. Positive value of coefficient of $X1$ (+1.17) indicates direct relationship of *C. reflexa* extract concentration and %CDR, that is, shows that higher extract concentration was associated with enhanced drug release. Conversely, the coefficient for $X2$ (−7.00) signifies a negative correlation with %CDR, implying that higher Carbopol concentrations result in decreased drug release, possibly due to increased gel viscosity, which retards drug diffusion.

Effect of independent factors on viscosity(cP) (Y2)-surface response study

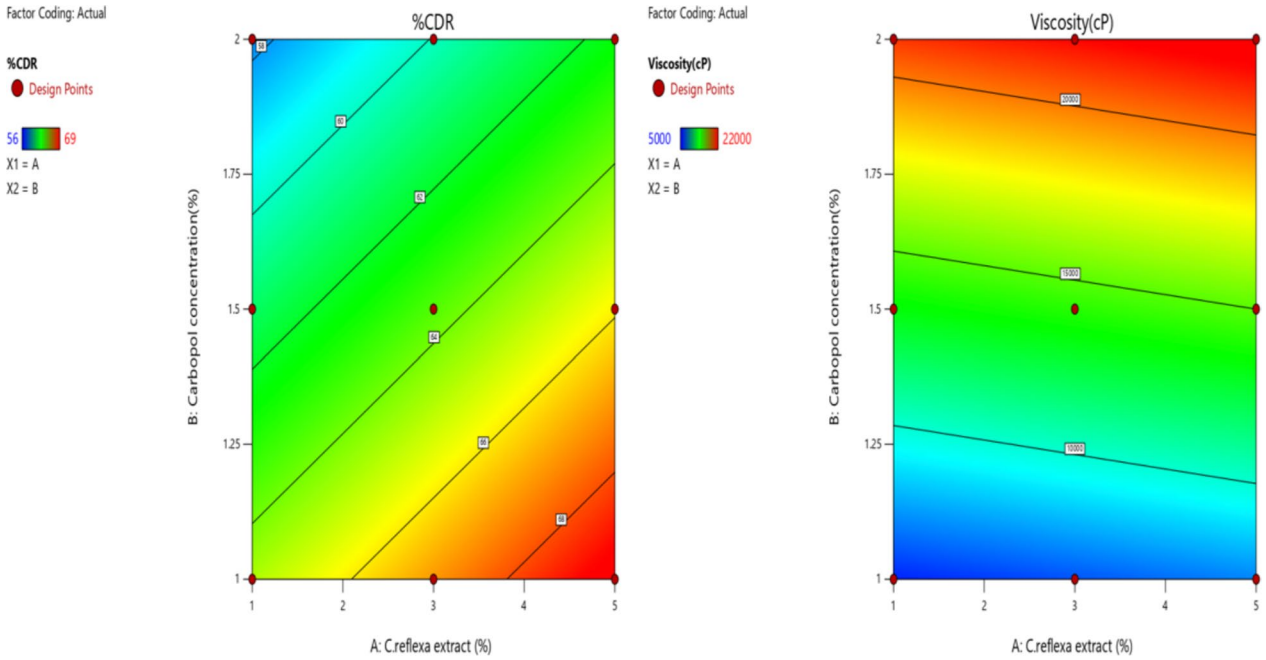
For the nine trials, the viscosity (cP) values were between of 5000–22000 cP.

$$Y2 = -10333 + 416 * X1 + 15500 * X2 \quad (2)$$

As per polynomial Eq. (2) and Counter plot (Fig. 1B), where the intercept (− 10,333) corresponds to the baseline estimate of viscosity when both independent factors are represented by their zero (or coded central) levels. The positive coefficient of $X1$ (+416) implies the direct relationship of *C. reflexa* extract concentration with viscosity, as that extract level increase resulted in moderate increase in formulation viscosity. The coefficient of $X2$ (+15,500) has demonstrated that Carbopol concentrations had a significant positive effect on viscosity. This was anticipated because the gelling agent is Carbopol which forms the hydrogel network, then the higher the content, the higher the viscosity of the formulation.

In the current study, 3^2 factorial designs in response surface methodology (RSM) in the form of point prediction methodology have been employed to optimize formulation of *C. reflexa* extract-loaded hydrogel. The composition of formulation of *C. Extract* (1.68%) and concentration of Carbopol (1.46%) complied the specification of the best formulation.

The optimized batch exhibited an % CDR ($Y1$) of 64%, Viscosity Cp ($Y2$) 15,164 cP, and desirability was 1.0. Table 8 presents the response of the optimized formulation of hydrogel formulation. All the observed near to value responses were optimally predicted by Design Expert software version 11.



A) Response surface plot of %CDR.

B) Response surface plot of Viscosity (cP).

Fig. 1 **A** Response surface plot of %CDR. **B** Response surface plot of viscosity (cP)

Table 8 Check point analysis of optimized batch

	Independent factor		Dependent factor	
	Cuscuta extract (%)	Carbopol concentration (%)	% CDR	Viscosity (Cp)
Predicted response	1.68	1.46	62.28	14,868
Actual response	1.68	1.46	64	15,164

Table 9 Characterization result of optimized batch of hydrogel formulation

Evaluation parameters	Optimized formulation
Color	Brown opaque
Texture	Smooth
Spreadability (%)	66
Viscosity (cP)	14,872
pH	6.32
Extrudability (cm)	14.2
Drug content (%)	94.3

Characterization of optimized batch of hydrogel formulation

The optimized *C. reflexa* hydrogel was tested based on the parameters listed in the methodology, and the results are summarized in Table 9.

Visual inspection of formulation

On visual examination, the hydrogel was a brown solid opaque with even smooth texture. No clumping, phase separation, or in consistencies were found which indicate good physical stability.

Determining pH

The pH of the formulation was registered 6.32, which is within the level of normal skin pH (4.5–7).

Spreadability test

The spread test gave a score of 66%, which can be considered a measure of how gel can be spread on top of the skin with ease and uniformity.

Determination of viscosity

The optimized hydrogel was tested, and the viscosity was determined to be 14,872 cP. The value indicates that the formulation had a balance between firmness (to hold its structure) and smooth application which is preferable with topical delivery systems.

Determination of drug content

The percentage weight of the drug was determined as 94.3%, in which there were no clumps or other issues of distribution and proper incorporation of *C. reflexa* extract in the matrix of the gel.

Determination of extrudability

The extrudability was tested at 14.2 cm indicating that the hydrogel could be easily extruded out of a collapsible tube using moderate force thus making it easy to use by the patients.

In vitro drug release studies for optimized hydrogel formulations

The samples were analyzed at 274 nm which corresponds to the λmax of *C. reflexa* containing apigenin. The results %CDR was found to be 64.00% for the optimized batch is *C. reflexa* extract-loaded gel containing apigenin demonstrated a controlled release over a period of 14 h, which was higher than that of the marketed extract-based formulation (Fig. 2). The optimized *C. reflexa* hydrogel exhibited a *T*50 of 9.7 h, whereas the marketed formulation did not achieve 50% release within the 14 h study period.

Ex vivo permeation study

The ex vivo drug retention and permeation study were performed with the *C. reflexa* extract-loaded hydrogel formulation and the marketed formulation. Drug permeation through the skin was 9% for *C. reflexa* hydrogel and 18% for the marketed herbal formulation, and drug retention in the superficial most layer of the epidermis and on the top layer of the skin was 48% and 32% in the CRE gel and the marketed formulation, respectively. (Fig. 3).

Animal study

Skin irritation studies

In order to assess the topical hydrogel's skin compatibility, a qualitative Draize protocol was performed on rat skin, and their effective drug delivery with no or slight skin irritation was pursued. Erythema and edema scores with respective formulations are shown in Table 10. Optimized formulation did not present any irritation (erythema and edema) at Primary Irritation Index (PII) value of 0.00, compared with Blank gel base group (PII 0.00), whereas the reference irritant (0.8% v/v formalin) caused mild to severe irritation.

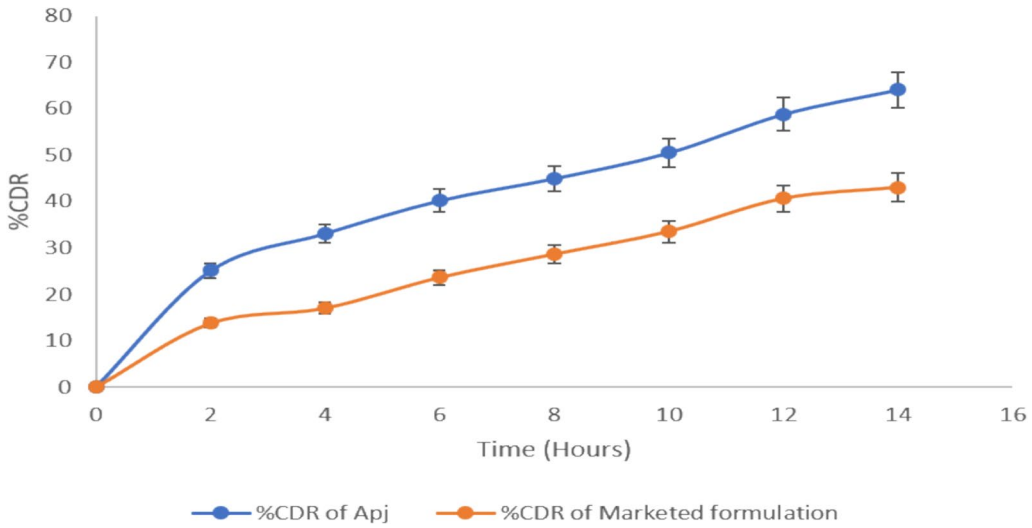


Fig. 2 Cumulative drug release studies of hydrogel and marketed formulation

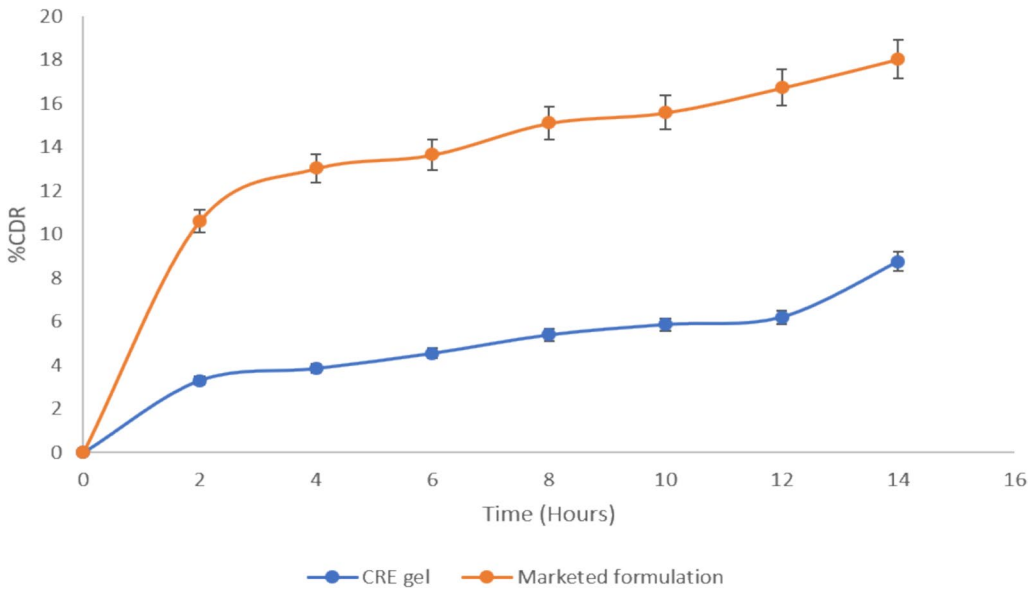


Fig. 3 Ex vivo drug permeation study of CRE hydrogel and marketed formulation

Table 10 Draize protocol-based scoring matrix used to assess the skin erythema and edema

Group of animals	Average draize score ($n=6$)					
	Erythema score (Time in hours)			Edema score (Time in hours)		
	24 h	48 h	72 h	24 h	48 h	78 h
Blank gel base (without extract)	0	0	0	0	0	0
0.8% v/v formalin solution	4	4	4	4	4	4
CRE gel	0	0	0	0	0	0

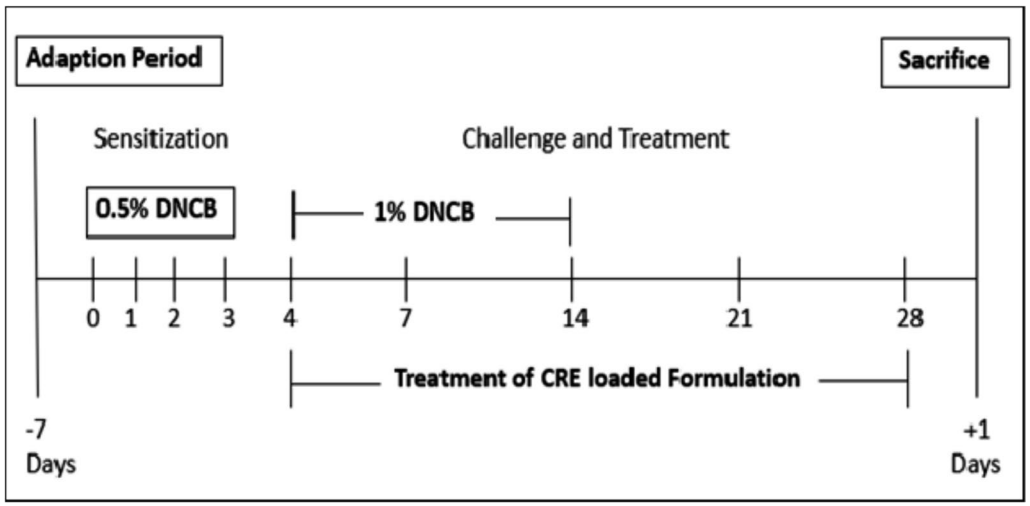


Fig. 4 Schematic representation of the experimental procedures for eczema mouse model study

In vivo efficacy studies in DNCB-induced eczema model

The effectiveness of the developed hydrogel was evaluated using a hypersensitive skin model of atopic dermatitis in mice where the DNCB solution was applied. Both the developed formulation and a standard marketed formulation were selected for comparative evaluation. Figure 4 provides a schematic representation of the experimental procedures used in the eczema mouse model study. Figure 5 shows the visual skin condition of the mice on day 7 (pre-treatment) and day 28 (post-treatment) together with the corresponding histopathological examination results. Histopathological examination of skin tissues (Figure 5 shows microscopic images of skin sections.) revealed that the disease control group exhibited severe epidermal hyperplasia, spongiosis, infiltration of inflammatory cells, and disruption of skin architecture. The optimized formulation yielded the significant beneficial effect as mild epidermal hyperplasia was evidenced, inflammatory cells infiltration was decreased, and skin structure was preserved that was similar with the positive control group. The placebo hydrogel group demonstrated marginal recovery versus the disease control group. The histological analysis of the DNCB-induced dermatitis model shows considerable variations between experimental groups. In the normal group, a normal pattern of tissue structure was observed with distinct epidermis, dermis, and hypodermis layers and few inflammatory cells.

In sharp contrast, the DNCB controls exhibited marked inflammatory responses: prominent erythema, edema, and crust formation, as well as typical histopathologic findings of epidermal hyperplasia, disturbed tissue architecture, and large numbers of neutrophils and mast cells. The cellular changes similarly mediate the pathophysiology of eczema, such that, neutrophils constitute the mechanism of involvement for acute inflammation, whereas mast cell degranulation results in increased vascular permeability and edema. The macroscopic findings of DNCB-treated animals are a direct mirror of the microscopic changes describing not only local tissue damage but also local immune response to the sensitizing agent. Both treatment regimens had a profound therapeutic effect, albeit with distinct differences. The treated group revealed remarkable recovery of inflammation both grossly and microscopically, with marked suppression of epidermal hyperplasia and a great decrease in neutrophilic infiltration and mast cell degranulation, suggesting silencing of major inflammatory mediators. Likewise, the marketed formulation group demonstrated some improvement as well, but histologic evaluation showed moderately greater inflammatory cellular still residual domain compared with the treatment and studied intervention group. The more moderate performance of the CRE gel in controlling the local and systemic inflammation is manifested in the less complete restoration of tissue architecture and greater inflammation cell infiltration. This suggests that the gel has less anti-inflammatory effects due to more abuse of neutrophils, mast cells, and other critical cells of Eczema pathophysiology and inflammation in various milieu more intense active suppressor of inflammation's action. These integrated histopathological results prove that although both formulations sought to relieve DNCB inflammation, the experimental CRE gel seemed to have the more profound impact on inflammatory cells and tissue restoration in a passive mode.

After 3-days sensitization phase with 0.5% DNCB, the mice demonstrated marked increase in skin dryness and inflammation relative to the unsensitized group.

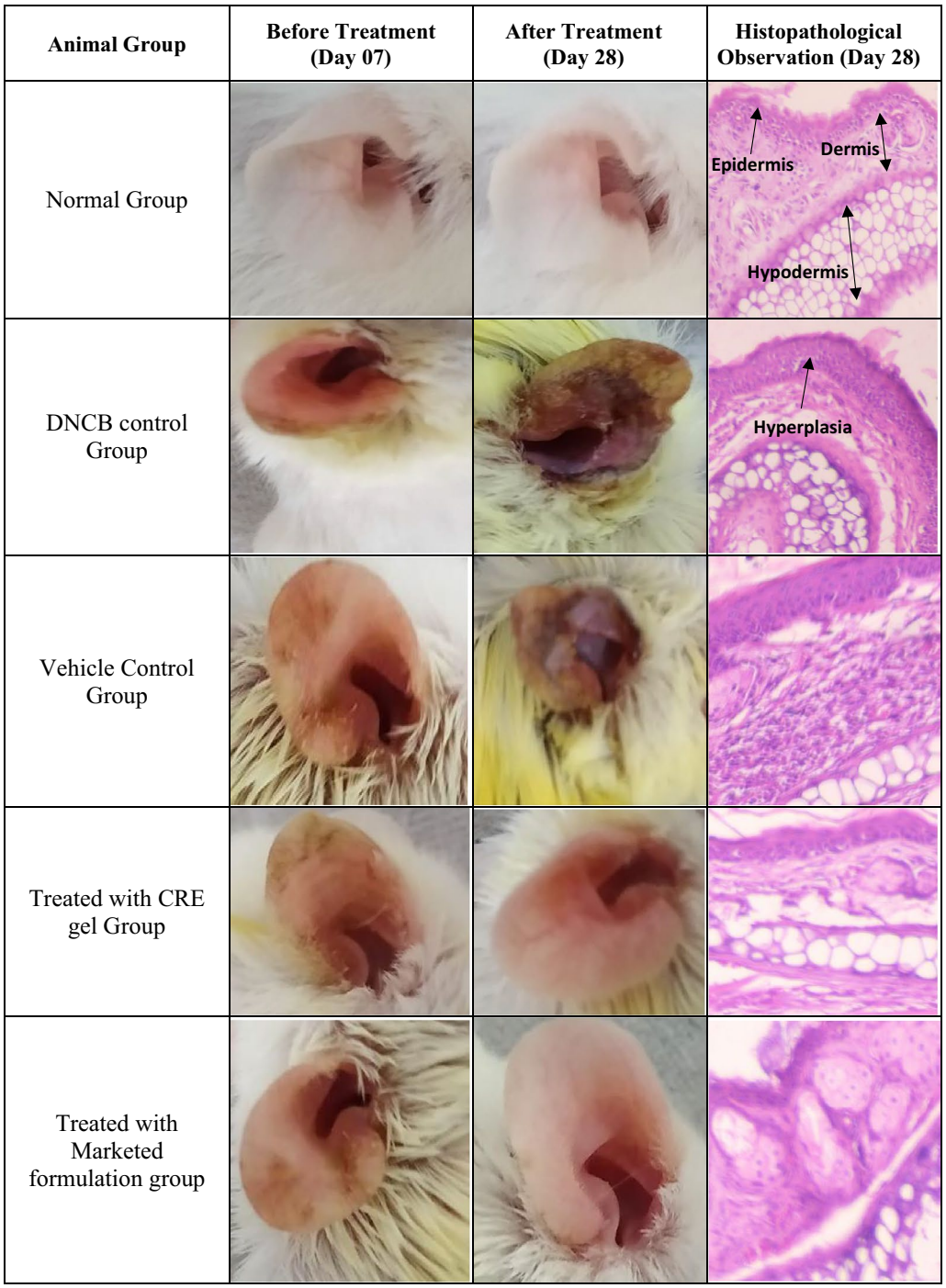


Fig. 5 Images depicting the auricular region of mice on day 7 (pre-treatment) and day 28 (post-treatment) together with the corresponding histopathological evaluation with optimized and commercial formulations

During the subsequent exposure phase, hallmark signs of eczema, including erythema, diffuse hemorrhage, edema, erosion, and lichenification, were most pronounced in the DNCB group. Compared with untreated eczema controls, application of the hydrogel formulation achieved greater clinical improvement.

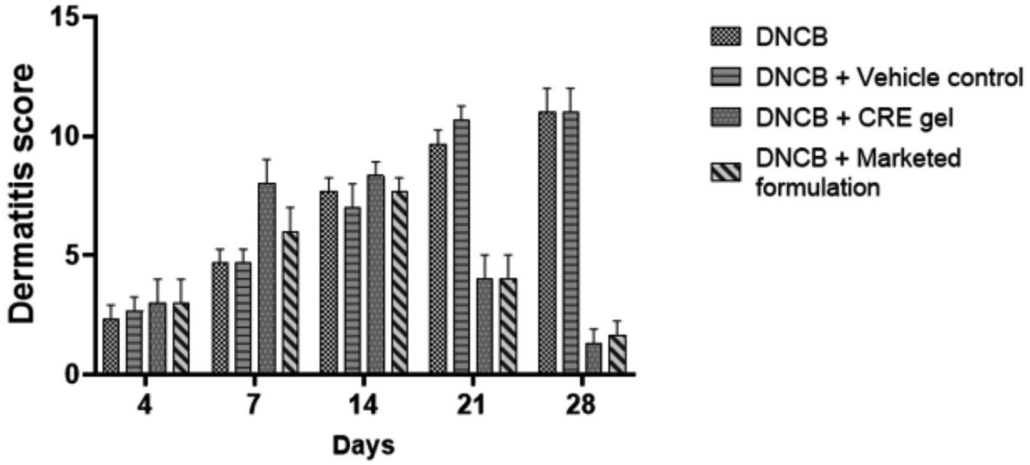


Fig. 6 Dermatitis scoring comparison among treated and untreated groups

Evaluation of eczema severity

The impact of several diverse gel formulations on DNCB-triggered skin inflammation was examined by keeping an eye on the dermatitis scores throughout a 28-day period, as shown in Fig. 6. The scores of the DNCB group were clearly rising, with the climax being reached on the 21st day, and staying quite high until day 28. On the other hand, both the *Cuscuta* gel and commercial formulation groups showed the effect and great improvement from the 14th day onward. The results by day 28, where the CRE gel had the symptoms significantly decreased, also indicates that the product was as effective as the marketed product, thus validating the outcome of ANOVA and post hoc solution.

The application of *C. reflexa* hydrogel on to mice skin was the transitions of the scores of erythema and edema and the general body conditions which, over the time of the experiment, were noted. During the entire evaluation period, increased erythema and edema scores and decreased general body condition scores were observed in the mice that received DNCB. These results are consistent with the known characteristics of DNCB-induced allergic dermatitis. The hydrogel can be considered an effective anti-inflammatory agent with significant wound-healing properties due to the extract. The removal of erythema and edema and the improvement of skin condition during the treatment period were observed.

In the ear thickness analysis showed in Fig. 7, in which the control group had the least ear thickness (0.2 mm) and the DNCB application showed the maximum inflammatory increase reaching nearly 1.7 mm. The treatments of both CRE gel and marketed formulation were efficient with the ear thickness reduced to around 0.7 mm, bringing about similar anti-inflammatory effect at the site of application. With a slight decrease in the thickness of the ear (1.5 mm), the DNCB + Vehicle group showed minimal improvement, thereby implying no substantial vehicle effect on local inflammation.

As the measurements of the spleen index Fig. 8 suggested, there was an additional corresponding change in systemic immune response that accompanied these local effects. About 0.33 was maintained by the control group's baseline spleen index, but DNCB exposure dramatically increased this to 0.83, indicating significant immune activation. The CRE gel has showed greater immunomodulatory properties owing to the reduction of the spleen index to around 0.50, while the marketed formulation showed moderate improvement (0.63). All these results indicate the efficacy of both formulations on DNCB-induced inflammation, with the experimental CRE gel leveraging greater synergistic impact on local as well as systemic inflammatory response compared to the commercial alternative.

Thus, the results indicate that the optimized *C. reflexa* hydrogel formulation efficiently ameliorated eczema symptoms by various mechanisms, i.e., decreased inflammation, immune response modulation, and improved skin barrier function. The observed anti-eczema effects may be attributed to the flavonoids and phenolic constituents in *C. reflexa*, known for their anti-inflammatory, antioxidant, and immune-modulatory activities.

Stability study

The long-term stability of the finalized hydrogel containing *C. reflexa* was assessed in relation to different storage conditions for a period of three months. The detailed findings are compiled in Table 11, which relates to the stability profile of the formulation over time.

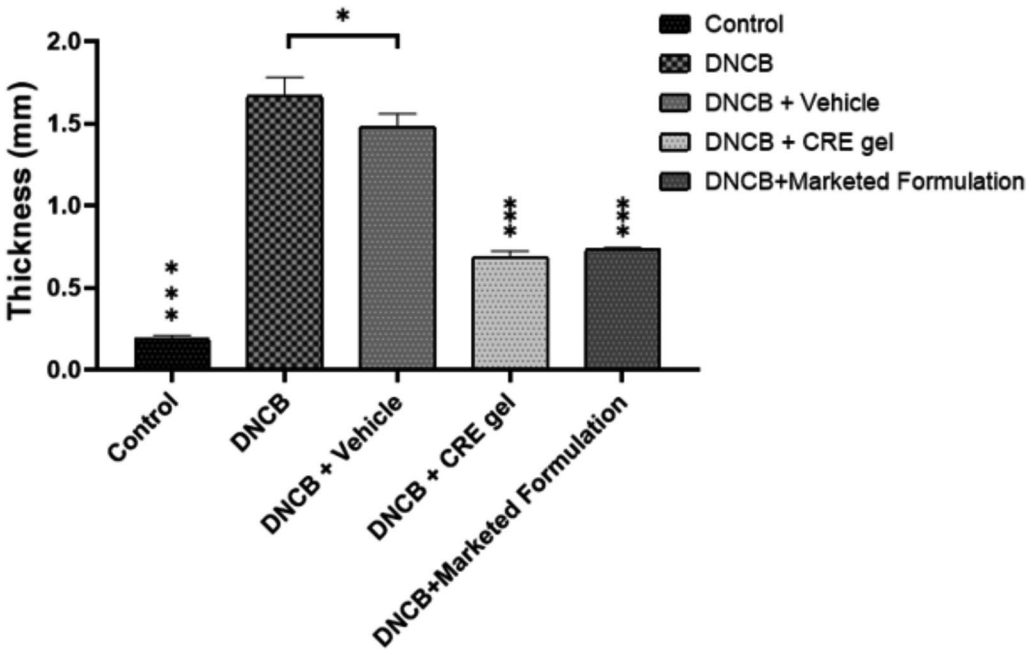


Fig. 7 Measurement of ear thickness in different treatment groups at day 29. Note: Each bar represents mean ± SD ($n = 6$). Statistical significance was determined by one-way ANOVA followed by Tukey's test; *$p < 0.05$, ***$p < 0.0001$

Discussion

The goal of the research was to design an effective topical hydrogel for the management of eczema using the plant extract of *C. reflexa*. The hydroalcoholic maceration of plant material yielded 21% extract in which the bioactive constituents were effectively extracted, and the phytochemical screening confirmed the presence of flavonoids, phenolic compounds, tannins, glycosides, and carbohydrates. The compounds have been extensively reported to exhibit antioxidant, anti-inflammatory, and wound-healing activities, which are most applicable in the treatment of eczema [37]. The pronounced content of flavonoids (84.50 mg/g QCT) and phenolics (35.56 mg/g GAE) underlines the phytochemical abundance of *C. reflexa* and confirms its possible therapeutic value. Moreover, the level of apigenin (7.69%) was also significant and, as it is proved, apigenin shows the anti-inflammatory, antioxidant, and immunomodulator activity, which could be one of the key factors for the bioactivity of the extract [38]. The initial hydrogel formulations created with different concentrations of Carbopol showed promising physicochemical characteristics such as smooth texture and no phase separation with pH readings in the healthy skin range of 6.3–7.2. Some extract and polymer concentrations clearly affected the performance of the gels and increased or decreased viscosity, as well as drug release, between the different formulations. 3^2 Factorial design along with regression analysis proved that the *C. reflexa* extract did enhance drug release while increased Carbopol concentration hindered it due to higher viscosity. Proportional to those two factors, viscosity increased as well with extract and polymer concentration. The optimized formulation with 1.68% extract and 1.46% Carbopol provided both viscosity and controlled sustained release yielding favorable therapeutic and application properties. The spreadability, extrudability, and uniform drug layering provided with the hydrogel ensured consistent dosing. The in vitro drug release profile of hydrogel showed controlled and sustained release over 14 h which is an advantage compared to the marketed formulation that requires frequent applications. *T*50 value confirm that the optimized formulation provided a comparatively faster and more efficient drug release profile than the marketed product. Ex vivo permeation studies showed that while the marketed formulation had higher permeation, the *C. reflexa* hydrogel had greater retention of the drug within the epidermal layers. Because it minimizes systemic absorption and guarantees targeted action at the site of inflammation, this localized delivery is especially advantageous for the treatment of eczema.

The Draize skin irritation study confirmed that the skin-friendly pH and the use of excipients, the optimized hydrogel was non-irritating. This is noteworthy because eczema patients have sensitive skin, so formulations should avoid further irritation. In vivo efficacy evaluation with the DNCB-induced eczema model showed that *C. reflexa* hydrogel treatment markedly decreased erythema, edema, epidermal hyperplasia, and inflammatory cell infiltration. Histopathological evaluation of the tissues also showed improvement in the architecture of the

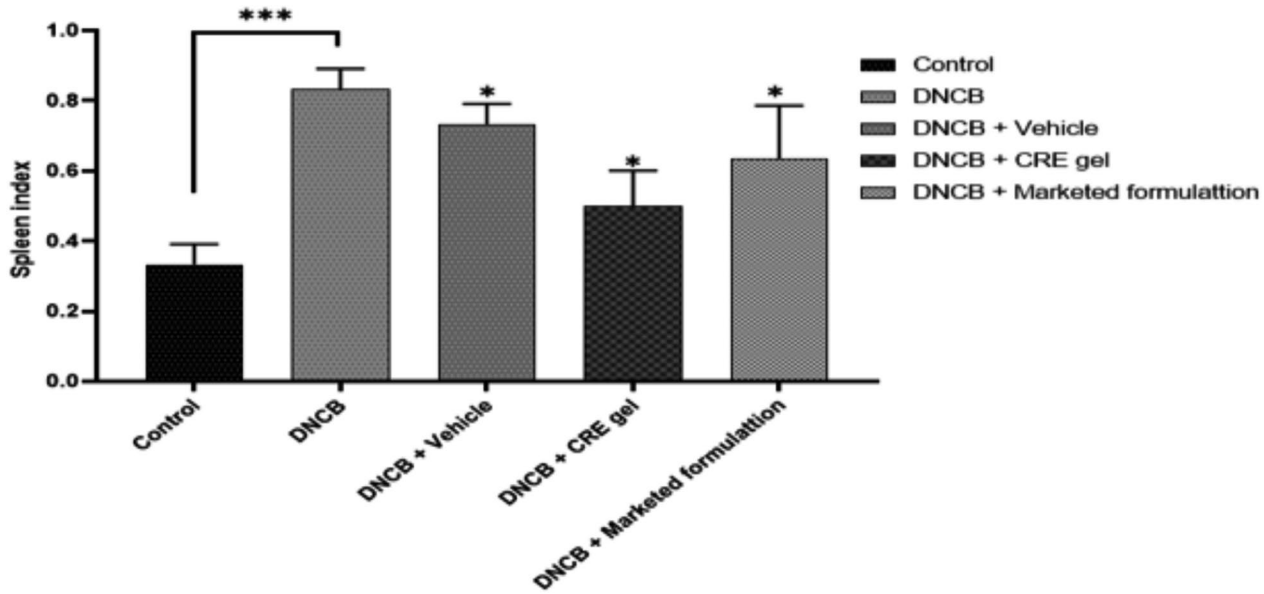


Fig. 8 Spleen index variation among mice receiving different formulations Note: Each bar represents mean ± SD ($n = 6$). Statistical significance was determined by one-way ANOVA followed by Tukey's test; *$p < 0.05$ versus DNCB + CRE gel and marketed formulation, ***$p < 0.0005$ versus Control and DNCB groups

tissues, and the effect was comparable to the marketed formulation. The formulation also highlighted significant anti-inflammatory and immunomodulatory effects as illustrated by progressive decrease in dermatitis scores, decreased ear thickness, and decreased spleen index. This was because of synergistic effects of flavonoids, phenolic compounds, and apigenin known to alter immune functions and inflammatory response. The stability test proved that the optimized hydrogel was physically and chemically stable in different storage conditions up to three months with no significant changes in viscosity, pH, spreadability, drug content, or drug release. Collectively, these results imply that the *C. reflexa*-based hydrogel is a potentially useful topical formulation in the management of eczema with sustained release, localized deposition on skin, safety, and therapeutic effect.

Conclusion

This study successfully developed and optimized a novel *C. reflexa* extract-based hydrogel for eczema management. The optimized formulation, containing Carbopol

Table 11 Stability profile of optimized *C. reflexa* hydrogel formulation

Evaluation parameter	Color	Viscosity (cPs)	Spreadability (g·cm/s)	Homogeneity	pH	Drug content (%)	%CDR
5 ± 3 °C (refrigerated)							
1 month	Opaque, Light Brown	15,020 ± 20	67 ± 3	Excellent	6.45 ± 0.05	95 ± 1.2	64 ± 2
2 months	Opaque, Light Brown	14,900 ± 10	69 ± 2	Excellent	6.37 ± 0.04	94 ± 1.5	63 ± 2
3 months	Opaque, Light Brown	14,800 ± 15	72 ± 3	Excellent	6.29 ± 0.06	94 ± 1.4	62 ± 2
25 ± 2 °C with 60 ± 5% RH							
1 month	Opaque, Brownish	14,500 ± 20	68 ± 2	Excellent	6.34 ± 0.05	93 ± 1.3	63 ± 2
2 months	Opaque, Brownish	14,281 ± 25	71 ± 3	Excellent	6.22 ± 0.04	92 ± 1.2	61 ± 2
3 months	Opaque, Brownish	14,000 ± 30	76 ± 4	Excellent	6.11 ± 0.05	90 ± 1.6	59 ± 3
40 ± 2 °C with 75 ± 5% RH							
1 month	Opaque, Dark Brown	14,100 ± 25	66 ± 2	Excellent	6.23 ± 0.06	91 ± 1.5	62 ± 2
2 months	Opaque, Dark Brown	13,950 ± 10	68 ± 2	Good	6.14 ± 0.05	89 ± 1.7	60 ± 2
3 months	Opaque, Dark Brown	13,750 ± 20	70 ± 3	Good	6.10 ± 0.07	87 ± 1.8	58 ± 3

934, had the appropriate physicochemical characteristics and achieved sustained release of the drug. In vivo studies were conducted on this work which supported its eczema-relieving effect via anti-inflammatory, immunomodulatory and skin barrier repair effects. The formulation displayed a great safety profile, no skin irritation, and its characteristics were still stable after three months of different storage conditions.

Abbreviations

AD	Atopic Dermatitis
ANOVA	Analysis of Variance
C. reflexa	*Cuscuta reflexa*
CRE	*Cuscuta reflexa* Extract-based formulation
CV	Coefficient of Variation
COX-2	Cyclooxygenase-2
Cps	Centipoise
DNCB	2,4-Dinitrochlorobenzene
µg	Microgram
mg	Milligram
mL	Milliliter
min	Minute/s
PG	Propylene glycol
PBS	Phosphate buffer solution
OECD	Organization for Economic Co-operation and Development
IAEC	Institutional Animal Ethics Committee
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals

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Author contributions
Author Rushil Shah performed the entire research work and prepared the manuscript. Nagja Tripathi and Shital Butani provided guidance and supervision throughout the research activities, and all authors read and approved the final manuscript.

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Data availability
All data generated and analyzed during this study are presented within this article. Additional data can be obtained from the corresponding author upon reasonable request. Studies involving plants must include a statement specifying the local, national or international guidelines and legislation, and the required or appropriate permissions and/or licenses for the study not applicable.

Declarations

Ethics approval and consent to participate
The developed form formulation was tested for skin irritation and in vivo efficacy study using Sprague–Dawley rats and ICR mice. The study started only after obtaining the approval from the Institutional Animal Ethics Committee (IAEC) as well as the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under registration number -883/PO/ReBi/S/05/CPCSEA.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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