RESEARCH PAPER

DoE Optimized Herbal Based Cosmetic Formulation for Pre Hair-Removal Treatment

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ABSTRACT
Background: Hair removal often results in pain, skin irritation, and risk of infection. Although techniques like laser and electrolysis are available, they are expensive, time-consuming, and may carry long-term side effects. The present study focuses on developing a herbal pre-care dusting powder to reduce discomfort during hair removal procedures.
Aim: Formulation and development of Pre hair removal treatment herbal powder.
Methods: The formulation incorporated Arnica montana and menthol as herbal anesthetic agents, kaolin as adsorbent and a talc–rice powder blend as flow enhancer. Preformulation studies (micromeritic and flow properties) were performed for each component. A 3×2 full factorial design was applied to optimize kaolin (X_1) and rice powder (X_2) against angle of repose (R_1), water absorptivity (R_2), and particle size (R_3). The optimized batch underwent in vivo testing for skin irritation (using digital RGB color picker tool) and analgesic activity (hot plate test).
Results: Angle of repose, %water absorptivity and particle size for DoE batches was found in the ranges of 25-32°, 58-80% and 37-51µm. The optimized formulation had desired AoR (28.97°), water absorptivity (68.82%) and particle size (42.75 µm). No skin redness and irritation was observed in animal models. Analgesic study model revealed significantly improved latency in paw licking (10.36 ± 0.54 s) and jumping (14.41 ± 0.69 s) compared to the control group (6.97 ± 0.46 s and 10.18 ± 0.31 s; $p < 0.05$).
Discussion: Herbal based optimized formulation demonstrated acceptable pharmaceutical and in vivo performance proving its competence for pre hair removal treatment care.
Conclusion: Herbal-based dusting powder can become a promising, safe and natural option for pre-treatment to reduce pain and irritation during hair removal procedures.
Keywords: *Herbal formulation, Dusting powder, Analgesic activity, Arnica montana, Factorial design, Skin irritation, Design of experiment.*
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INTRODUCTION

Hair is one of mammals' distinctive traits, serving a range of purposes such as protection from external forces, sebum production, apocrine sweat, and many others. However, unwanted hair growth is a common concern among teenage girls and women. Hair removal, commonly known as epilation or depilation, is the purposeful removal of body or scalp hair. Hair develops all throughout the body and varies in thickness and length across different populations. Hair grows more noticeable during and after adolescence, and men typically have thicker, more prominent body hair than women[1]. Both men and women have noticeable body hair on their heads, brows, eyelids, armpits, genitals, arms, and legs. Hair removal may be practiced for cultural, fashion, aesthetic, hygiene, sexual, medical, sports, military, or certain religious reasons. Forms of hair removal have been practiced in almost all human cultures since at least the Neolithic era. The methods used to remove hair have varied in different times and regions[2-6].

Hair removal methods

There are mainly two types of hair removal methods: Depilation and Epilation methods. Depilation removes hair only from the surface of the skin, leaving the root intact. Hair grows back faster within several hours to several days. Depilation methods include shaving and depilatory products. Epilation removes hair from the root, resulting in longer lasting smoothness. Since the hair is pulled out from the follicle, regrowth is slower. Epilation includes waxing, threading, tweezing, epilators, laser and electrolysis. Each method has their own pros and cons. Depilation methods are painless but hair regrowth occurs within 2-3 days. Waxing and threading are effective methods but are painful

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and cause discomfort and irritation. Advanced methods like laser and electrolysis may lead to long term risks[7,8].

Pre-treatment formulation

The pre-treatment hair removal dusting powder is a specially designed formulation to enhance comfort during the hair removal process. It features *Arnica montana* hydroalcoholic flower extract, rich in sesquiterpene lactones, known for their natural analgesic properties[9-11]. To further amplify the numbing effect, menthol is incorporated, providing a soothing, cooling sensation[12,13]. This herbal-based formulation helps reduce discomfort, preparing the skin for a smoother and less painful hair removal experience. Applied immediately before the procedure, it serves as an effective pre-treatment solution for improved skin comfort and tolerance.

The primary objectives of pre-treatment formulation were, to minimize the pain and discomfort and prepare the skin suitable for hair removal process, characterization of the same using appropriate evaluation parameters.

2. MATERIALS & METHODS

Materials

Arnica montana (Herbal analgesic agent) hydroalcoholic extract was obtained as a gift sample from Extroil Naturals (Ahmedabad, India) along with certificate of analysis, Menthol was purchased from R B remedies (Ahmedabad, India), Kaolin was purchased from Kalpana chemicals (Ahmedabad, India), Kieselguhr was purchased from Nupur internationals (Ahmedabad, India), Rice powder was purchased from Gujarat Traders (Gandhi Dham, India), Talc was purchased from Shree Vallabh Industries (Gandhinagar, India). All the materials used were of analytical grade.

Methods

Pre-formulation studies of Materials

1. Derived properties

The compressibility index measures a solid's capacity to be compressed. The powder (25 g) was placed into a measuring cylinder of a tapped density apparatus, and the bulk volume, V1, was recorded. After 100 taps, the tapped volume, V2, was measured. The bulk and tapped densities were computed as mentioned in Eq (1) and (2).

$$Bulk\ Density = Mass/Volume \quad \text{Eq (1)}$$

$$Tapped\ Density = Mass/Tapped\ Volume \quad \text{Eq (2)}$$

The Hausner ratio and Carr index (CI) were calculated using the data of bulk density and tapped density respective for the materials according to the Eq (3) and (4).

$$Hausner\ ratio = Tapped\ Densit/Bulk\ Density \quad \text{Eq (3)}$$

$$Carr's\ Index = \frac{Tapped\ Density - Bulk\ Density}{Tapped\ Density} * 100 \quad \text{Eq (4)}$$

The flow property of the powder was examined by measuring its angle of repose using the fixed funnel method mentioned in Eq (5).

$$Angle\ of\ repose\ \Theta = \tan^{-1} h/r \quad \text{Eq (5)}$$

Where, Θ = angle of repose (◦); h = height of the pile (cm); r = radius of the pile (cm).

The mean angle of repose was computed after making triplicate determinations[14].

2. Particle size analysis

Particle size analysis of the powder was conducted using an optical microscopy technique, as per procedures outlined in standard pharmacopeial references. A representative sample of the powder was lightly spread on a clean glass slide. A drop of suitable dispersing medium (such as glycerin or liquid paraffin) was added to minimize particle agglomeration and ensure uniform dispersion. The sample was then covered with a cover slip and observed under a compound microscope at 400× magnification. Prior to measurement, the microscope was calibrated using a stage micrometer to correlate the eyepiece micrometer divisions with actual distances (in microns). Particles size was measured using the calibrated eyepiece micrometer. A minimum of 100 individual particles were randomly selected and their size recorded. The data were expressed in terms of particle size. The average particle size was calculated using the formula mention in Eq (6).

$$Average\ particle\ size = \Sigma d/n \quad \text{Eq (6)}$$

Where, *d* is the size of each particle and *n* is the number of particles measured.

3. WATER ABSORPTIVITY

The hydration capacity (water retention capacity) was assessed using the previously described method[15]. One gram of powder was placed in a centrifuge tube and coated with 10 millilitres of water. The tube was shook intermittently for two hours and then let to stand for 30 minutes. The sample was then centrifuged for 10 minutes at 3000 rpm. Decant the supernatant and weigh the powder after water uptake and centrifugation, was determined as mentioned in Eq (7).

$$Water\ Absorptivity\ Index(\%) = (wt.of\ wet\ residue/wt.\ of\ sample) * 100 \quad \text{Eq (7)}$$

4. SWELLING CAPACITY

The powder's swelling capacity was determined using the previously reported method[15]. The tapping volume occupied by 1 gram of powder Vx was observed. The powder was then dispersed in 85.0 ml of water and the volume was increased to 100 ml with additional water. After 24 hours of standing, the volume of sediment, Vy, was calculated.

The swelling capacity was computed as shown in Eq (8).

$$Swelling\ capacity = \frac{Vy}{Vx} \quad \text{Eq (8)}$$

The mean of the three determinations was calculated.

5. pH

The pH of the 1% aqueous dispersion was obtained by an electronic pH meter.(3020 pH meter, Jenway, UK)[15].

Preparation of Screening Batches of pre-treatment formulation

According to the results obtained from pre-formulation evaluation of ingredients, screening batches (S1-S13) were formulated and their evaluation results obtained are listed in Table 6. The powdered materials were sieved with #40 mesh, weighed properly, then blended geometrically to ensure consistent mixing[16]. Screening batches formulations are shown in **Table 1**.

Table 1 Formulation of Screening batches

Ingredients (%)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Arnica extract	5	5	5	5	5	5	5	5	5	5	5	5	5
Menthol	2	2	2	2	2	2	2	2	2	2	2	2	2
Kaolin	10	10	10	10	10	10	-	-	-	-	-	-	5
Bentonite	-	-	-	-	-	-	10	10	10	10	10	10	5
Talc	q.s.	-	-	-	-	-	q.s.	-	-	-	-	-	q.s.
Rice powder	-	q.s.	-	-	-	-	-	q.s.	-	-	-	-	-
Rice starch	-	-	q.s.	-	-	-	-	-	-	-	-	-	-
Maize starch	-	-	-	q.s.	-	-	-	-	-	-	-	-	-
Potato starch	-	-	-		q.s.	-	-	-	-	-	q.s.	-	-
Kieselguhr	-	-	-	-	-	q.s.	-	-	-	-	-	q.s.	-

Preparation of Trial Batches of pre-treatment formulation

According to the results obtained from evaluation of screening batches, trial batches (T1-T4) were formulated shown in **Table 2**.

Table 2 Formulation of Trial batches

Ingredients (%)	T1	T2	T3	T4
Arnica extract	5	5	5	5
Menthol	2	2	2	2
Kaolin	10	10	10	10
Talc	10	40	-	-
Rice powder	-	-	10	40
Kieselguhr	q.s.	q.s.	q.s.	q.s.

Optimization of pre-treatment formulation by DoE

Optimizing formulation was derived by applying 3^2 full factorial design on independent factor using DESIGN® EXPERT 7.0 demo version software (STAT-EASE). Concentration of kaolin and rice powder were selected as independent variables as X1 and X2 respectively and studied at 3 levels each[17]. Evaluation parameters such as Angle of Repose (R1), Water absorptivity (R2) and Particle size(R3) were selected as a dependent variable (Responses). In this design, two factors were studied at three levels and experimental trials were performed at all 9 possible combinations. Two checkpoint batch were also formulated to validate the validate software derived models. Formulation of Factorial and checkpoint batches are shown in **Table 3**.

Table 3 Formulation of Factorial Batches pre-treatment formulation

Ingredients (%)	**Factorial Batches**									**Checkpoint Batches**	
	F1	F2	F3	F4	F5	F6	F7	F8	F9	CHK1	CHK2
Arnica extract	5	5	5	5	5	5	5	5	5	5	5
Menthol	2	2	2	2	2	2	2	2	2	2	2
Kaolin	5	5	5	10	10	10	15	15	15	6.65	12.45
Rice powder	10	25	40	10	25	40	10	25	40	31.3	23.95
Kieselguhr	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Characterization of Pre-treatment formulation

Pre-formulation studies were performed for all trial and factorial batches as mentioned above.

Animal study

In vivo evaluations were carried out to determine the dermal safety and functional efficacy of the developed topical pre-treatment formulation, focusing on its potential to cause skin irritation and its topical analgesic activity. These studies were essential to confirm the formulation's compatibility with skin and its intended anesthetic effect before progressing toward human application. All experimental procedures complied with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), and the protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of RK University, Rajkot, under proposal number RKCP/CO1/Re/24/154.

Skin Irritation test

Experiment was carried out using 24 adults (either sex) to test for skin irritation and analgesic activity of test formulation. They were kept carefully following an acclimation period of 7 days to ensure their suitability for the study. Animals were kept with environmental conditions set to a temperature of 25 ± 2º C, a humidity of 60-90% RH and a 12-h light / 12-h dark cycle. Grouping of animals has been done as shown in **Table 4**.

Table 4 Grouping of animals for skin Irritation test

Sr. No.	Group of Animals	Treatment	No. of Animals
1	Normal control	Vehicle applied on dorsal surface of rats	6
2	Negative control	Sodium Lauryl Sulfate applied on dorsal Surface of rats	6
3	Positive control	Talcum Powder applied on dorsal Surface of rats	6
4	Test control	Pre-treatment formulation applied on dorsal Surface of rats	6
Total=24			

The irritancy potential of the topical powder formulation was evaluated using a rat model. Prior to application, the dorsal surface of each rat was carefully shaved to expose an area of approximately 2 × 2 cm. The animals were then left undisturbed for 24 hours to allow any inflammation or redness caused by shaving to subside. On the following day, a small quantity (pinch) of the powder was uniformly applied to the shaved area of each animal.

Observations for signs of erythema (redness) were carried out visually at predetermined time intervals: 0, 5, and 10 minutes post-application.

Photographic documentation of the application site was performed at each time point. The images were then analyzed using a digital color analysis tool (Color Picker) to quantify the redness. The intensity of redness was assessed by extracting Red-Green-Blue (RGB) values from the photographed areas, enabling objective comparison between the SLS-treated group and the group treated with the test (pre-treatment) formulation.

This method provided a semi-quantitative evaluation of skin irritation based on colorimetric differences in erythema intensity[18–22].

Topical analgesic activity test

Eighteen adult wistar rats (n = 3 per group) were used in the study. The animals were housed under standard laboratory conditions with free access to food and water. Grouping of animals has been done as shown in **Table 5**.

Table 5 Grouping of animals for Analgesic test

Sr. No.	Group of Animals	Treatment	No. of Animals
1	Normal control	Vehicle applied on Four Paws of rats	6
2	Positive control	Lidocaine topical gel 2% applied on Four Paws of rats	6
3	Test control	Pre-treatment formulation applied on Four Paws of rats	6
Total=18			

The Eddy's Hot Plate Method was used to evaluate the analgesic activity. The hot plate was maintained at 55 ± 1°C, and each animal was placed individually on the heated surface. A small amount of the assigned formulation was applied topically to the plantar surface of all four paws of each animal. The paw licking latency and jumping response time were recorded at three-time intervals: 0 min (baseline), 5 min, and 15 min post-application. A cut-off time of 180 seconds was observed to avoid tissue damage. Observations were recorded for both behavioural responses: Paw licking latency (in seconds), Jumping response latency (in seconds)[23,24].

Stability study

The produced formulations were tested for stability according to ICH requirements by storing them at various temperatures and humidity levels for six months[25]. The packed formulation was examined at various temperatures, including room temperature, 35°C, and 40°C. Physical qualities such as color, smell, and texture[26].

RESULT & DISCUSSION

Pre-formulation studies of materials

Preformulation studies were conducted to evaluate the flow properties of each individual component used in the formulation shown in **Table 6**.

Table 6 Preformulation study of Ingredients

Ingredients	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Carr's Index (%)	Hausner ratio	Angle of Repose (°)	Average Particle size (µm)
Arnica extract	0.5	0.58	13.7	1.16	24.22	30.6
Menthol	0.33	0.45	26.6	1.36	47.22	27
Kaolin	0.18	0.24	25	1.33	47.22	39.6
Bentonite	0.86	1.07	19.62	1.24	74.14	51.25
Talc	0.24	0.3	24.8	1.25	48.01	33
Rice powder	0.66	0.95	30.5	1.5	46.6	38.5
Rice starch	0.5	0.82	21.02	1.64	47.46	32.5
Maize starch	0.51	0.73	30.13	1.43	41.66	49.8

Potato starch	0.6	0.89	32.5	1.41	41.66	48
Kieselguhr	0.25	0.35	28.5	1.4	50.65	50
n=3						

Characterization of Screening batches

Trial batches S1-S13 were formulated and their evaluation results obtained are listed in **Table 7**.

Table 7 Evaluation of Screening batches

Trial batches	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Carr's Index (%)	Hausner ratio	Angle of Repose (°)	Average Particle size (µm)	Water absorptivity (%)
S1	0.44	0.52	15.38	1.18	32.61	42.5	12
S2	0.55	0.71	22.53	1.29	30.96	60	38
S3	0.55	0.76	27.63	1.38	34.21	43.7	42
S4	0.43	0.58	25.86	1.34	33.38	65	54
S5	0.52	0.66	21.21	1.26	34.99	46.25	62
S6	0.23	0.31	25.8	1.34	39.69	57.5	72
S7	0.43	0.55	21.89	1.27	39.69	57.5	28
S8	0.57	0.66	13.63	0.09	34.99	51.25	40
S9	0.55	0.68	19.11	1.23	37.23	57.5	44
S10	0.47	0.62	24.19	1.31	35.37	51.25	50
S11	0.48	0.62	22.58	1.29	39.69	58.75	60
S12	0.23	0.33	30.3	1.43	41.98	32.5	75
S13	0.45	0.55	18.18	1.22	41.34	53.75	45
n=3							

Evaluation of the screening batches was revealed that Kieselguhr showed excellent water absorptivity but poor flow properties, making it unsuitable as a sole component. In contrast, talc and rice powder demonstrated good flow behaviour. To achieve a balanced formulation, kieselguhr was combined with glidants like talc or rice powder to improve flow without compromising its adsorptive capacity. Based on these observations, Batches S1, S2 and S5 were found to be following majority of desired criteria. Therefore, pre-experimental batches (E1–E4) were prepared and evaluated with the aim of selecting a suitable glidant.

Characterization of Trial batches

Trial batches T1-T4 were formulated and their evaluation results obtained are listed in **Table 8**.

Table 8 Evaluation of Trial batches

Trial batches	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Carr's Index (%)	Hausner ratio	Angle of Repose (°)	Average Particle size (µm)	Water absorptivity (%)
T1	0.28	0.46	39.13	1.64	26.56	51	55
T2	0.31	0.57	45.61	1.83	23.26	49.5	40
T3	0.30	0.48	37.5	1.6	22.29	51	65
T4	0.42	0.6	30	1.42	20.8	55.5	80
n=3							

From the results of the experimental batches, it was observed that formulations containing rice powder (E3 and E4) exhibited both desirable properties—good angle of repose and satisfactory water absorptivity. Hence, rice powder was considered as the suitable glidant, and the following ingredients were finalized for the development of factorial batches: Arnica extract, Menthol, Kaolin, Rice powder, and Kieselguhr.

Optimization of Pre-treatment formulation using DoE

Responses of factorial batches were analysed using response surface methodology. ANOVA was applied to derive statistical model of each responses using DESIGN® EXPERT 7.0 demo version software (STAT-EASE). Composition of the 3^2 full factorial design investigated is shown in **Table 9**.

Table 9 Factorial Batches layout and responses

Batch code	**Coded Values**		**Factorial Responses**		
	X1 Kaolin (%)	X2 Rice powder (%)	R1 Angle of Repose (°)	R2 % Water absorptivity	R3 Particle size (µm)
F1	-1	-1	32.42	80	51.3
F2	-1	0	29.51	73	45.6
F3	-1	1	26.63	65	39.2
F4	0	-1	31.33	78	48.7
F5	0	0	28.76	71	43.4
F6	0	1	25.12	59	38.5
F7	1	-1	30.7	74	46.9
F8	1	0	27.42	67	42
F9	1	1	29.98	58	37.8
CHK 1	-0.67	0.42	28.24	68.51	42.39

CHK 2	0.49	-0.07	28.73	69.42	43
Translation of coded level in actual level					
Coded level			-1	0	1
X1 Kaolin (%)			5	10	15
X2 Rice powder (%)			10	25	40

Optimization data analysis

A 3^2 complete factorial design was used to determine the optimal batch based on specific parameters. ANOVA was used to analyze answers by a statistical model that included polynomial and interactive terms based on the data gathered for factorial batches. Full and reduced (significance level 5%) statistical model derived for responses R1(Angle of repose), R2 (% water absorptivity) and R3 (Particle size). are shown in **equation 8-10** respectively. Corresponding response surface plots and overly plot is shown in **figure 1.**

$$R1 = 29.10 - 1.05 * A - 310 * B + 1.27 * AB + 1.48 * A2B + 1.45 * AB2 \quad \text{Eq (8)}$$

$$R2 = 70.22 - 3.16 * A - 8.33 * B - 0.25 * AB + 0.16 * A2 - 1.33 * B2 \quad \text{Eq (9)}$$

$$R3 = 43.49 - 1.80 * A - 5.10 * B + 0.75 * AB + 0.27 * A2 + 0.67 * B2 - 0.20A2B + 0.35 * AB2 \quad \text{Eq (10)}$$

It was clear from Eq. (8) that while kaolin and rice powder individually reduced the angle of repose, their combination resulted in an increased value, suggesting a non-linear interaction influencing powder flow properties.

Eq. (9) revealed that kaolin and rice powder individually decreased water absorptivity, and their combination further reduced it, indicating a linear interaction affecting % water absorptivity.

Eq. (10) demonstrated that kaolin and rice powder individually reduced particle size, whereas their combination increased it, indicating a non-linear interaction influencing % particle size.

The analysis of the response surface graphs and corresponding mathematical models revealed a complex relationship between the independent variables (X1: Kaolin and X2: Rice powder) and the responses. Individually, X1 and X2 exhibit positive and negative coefficients respectively, suggesting that they have opposing effects on the measured responses. However, when combined, the interaction term (X1*X2) produces effects that can be either positive or negative, depending on the specific combination. This highlights the non-linear and intricate interplay between Kaolin and Rice powder, making it difficult to establish a direct correlation between polymer amounts and the responses (Angle of repose, % water absorptivity, particle size).

To determine the optimal formulation region within the design space, target values for all responses were input into optimization software. An overlay plot was generated, illustrating the optimized region as a yellow-coloured area (Figure 1). This plot aids in identifying the ideal balance of Kaolin and Rice powder to achieve the desired tablet properties.

X axis: Amount of Kaolin, Y axis: Amount of Rice powder

Figure 1 Response surface plots and overlay plot for optimization of Pre-treatment formulation

Model validation and optimized batch selection

Derived models were validated using two checkpoint batches: CHK1 and CHK2. **Table 10** shows comparative difference between predicted and experimental values of responses obtained for both checkpoint batches. Less than 5% deviation in the values proves that derived models were valid considering deviation of 5% between actual values and model predicted values.

Table 10 Validation of derived models by checkpoint batches

Checkpoint batches	Response	Predicted value	Actual value	%Error
CHK 1	R1	28.24	28.84	2.12
	R2	68.51	65.87	3.85
	R3	42.39	43.75	3.20
CHK 2	R1	28.73	27.93	2.80
	R2	69.42	70.44	1.47
	R3	43	42	2.32

To derive the optimized levels of the variables, a desirability function was used. Out of several levels suggested by the software falling under the optimized region of the overlay plot, levels with highest desirability were selected as optimized one. Optimized levels, their corresponding actual levels and final composition of the pre-treatment formulation are given in **Table 11 and 12** below. Optimized batch was formulated and evaluated in order to validate the derived formulation. All the parameters were falling under acceptable criteria range.

Table 11 Optimized formulation of cosmetic powder for pre hair removal treatment

Factor	Coded value	Actual value
Kaolin	-0.11	9.45%
Rice powder	0.18	27.7%

Table 12 Formulation of optimized batch of Pre-treatment powder

Composition	Quantity (%)
Arnica extract	5
Menthol	2
Kaolin	9.45
Rice powder	27.7
Kieselguhr(q.s.)	100

Stability study

The stability of the prepared topical powder formulation was assessed over a period of six month by storing samples at room temperature, 35 °C, and 40 °C. Physical parameters such as color, odor, and texture were evaluated at specified intervals to monitor any changes indicating physical instability. The stability data shown in **Table 13**.

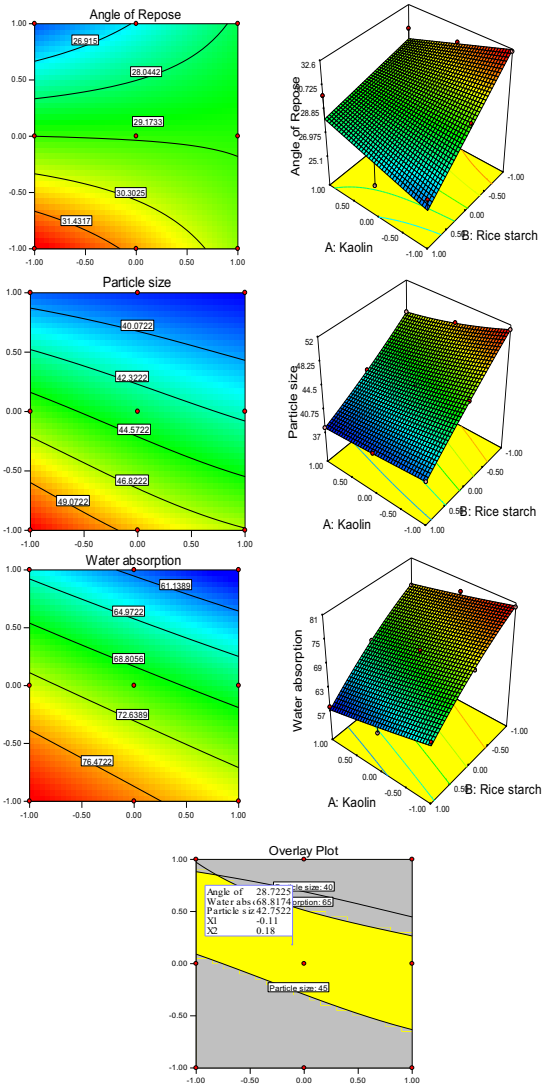


Table 13 Stability study of optimized batch

Storage condition	Day 0	1 month	3 months	6 months
25°C±2°C/ 60%±5% R.H.	Light brown, herbal odor, smooth texture	No change	No change	No change
30°C±2°C/ 65%±5% R.H.	Light brown, herbal odor, smooth texture	No change	No change	No change
40°C±2 °C /75%±5% R.H.	Light brown, herbal odor, smooth texture	No change	No change	No change

None of the metrics changed significantly under any of the storage settings examined.There were no signs of caking, clumping, or discoloration, suggesting that the formulation remained physically stable under both ambient and accelerated conditions. These findings confirms that the selected combination of ingredients were compatible and contribute to a stable formulation suitable for long term storage.

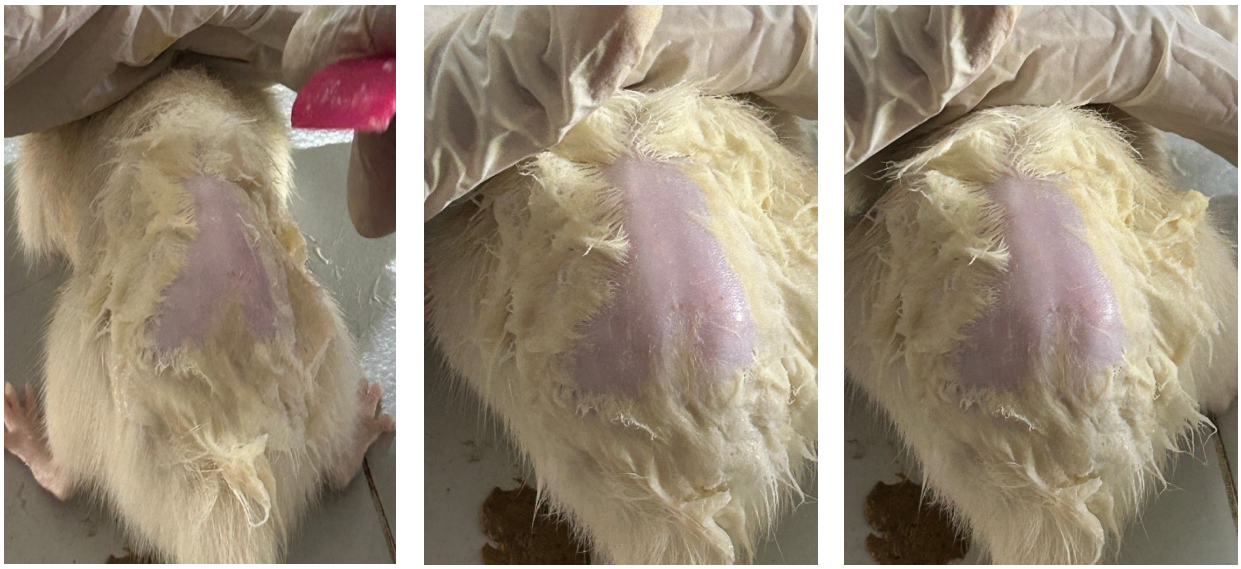
Animal study

Irritancy test

The skin irritancy potential of the test formulation was evaluated using a rat model and quantified by digital image analysis. Images of the treated dorsal skin were captured at 0, 5, and 15 minutes post-application, and the redness intensity was measured using a digital color picker tool, reported in RGBA (Red, Green, Blue, Alpha) values. The focus was on changes in the red (R) component, which correlates with erythema. The results are shown in **Table 14** and **Figure 2,3,4 and 5**.

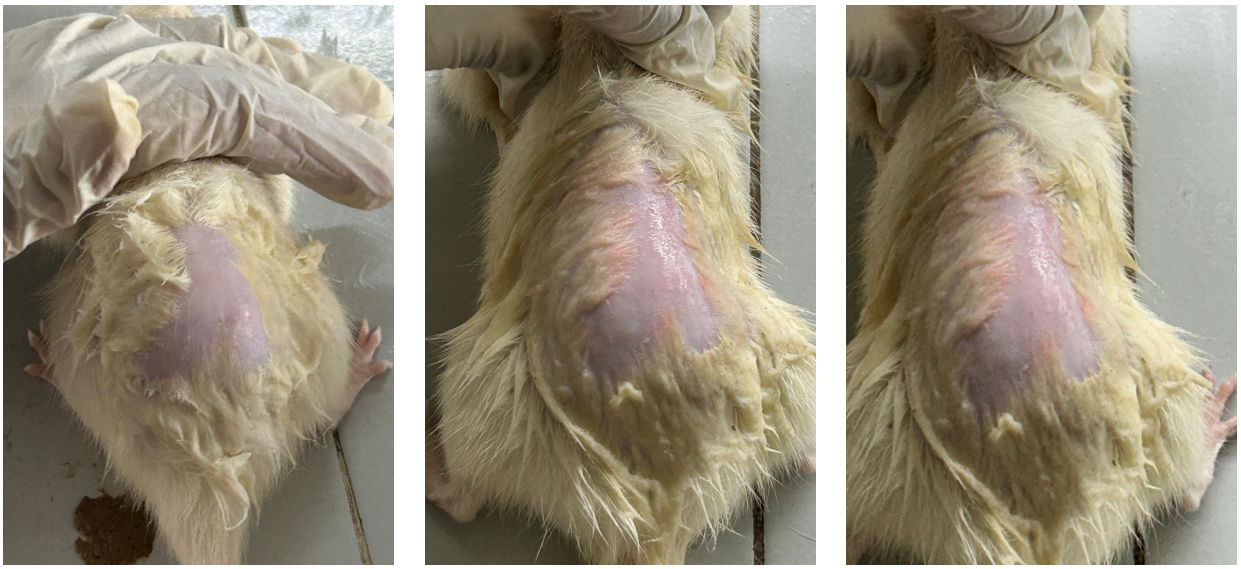
Table 14 RGBA values for irritancy test

Group of Animals	Time Interval(min)	R value	G value	B value	A value
Normal control	0	165	147	125	255
	5	191	164	169	255
	15	180	155	158	255
Negative control	0	182	160	162	255
	5	255	248	250	255
	15	208	181	186	255
Positive control	0	174	146	143	255
	5	177	145	150	255
	15	173	149	149	255
Test control	0	114	100	97	255
	5	115	98	90	255
	15	124	104	106	255



2(A) 2(B) 2(C)

Figure 2 Irritancy test on Normal control group at 0 minute **2(A),** 5 minutes **2(B)** and 15 minutes **2(C)** time intervals respectively



3(A) 3(B) 3(C)

Figure 3 Irritancy test on Negative control group at 0 minute **3(A),** 5 minutes **3(B)** and 15 minutes **3(C)** time intervals respectively

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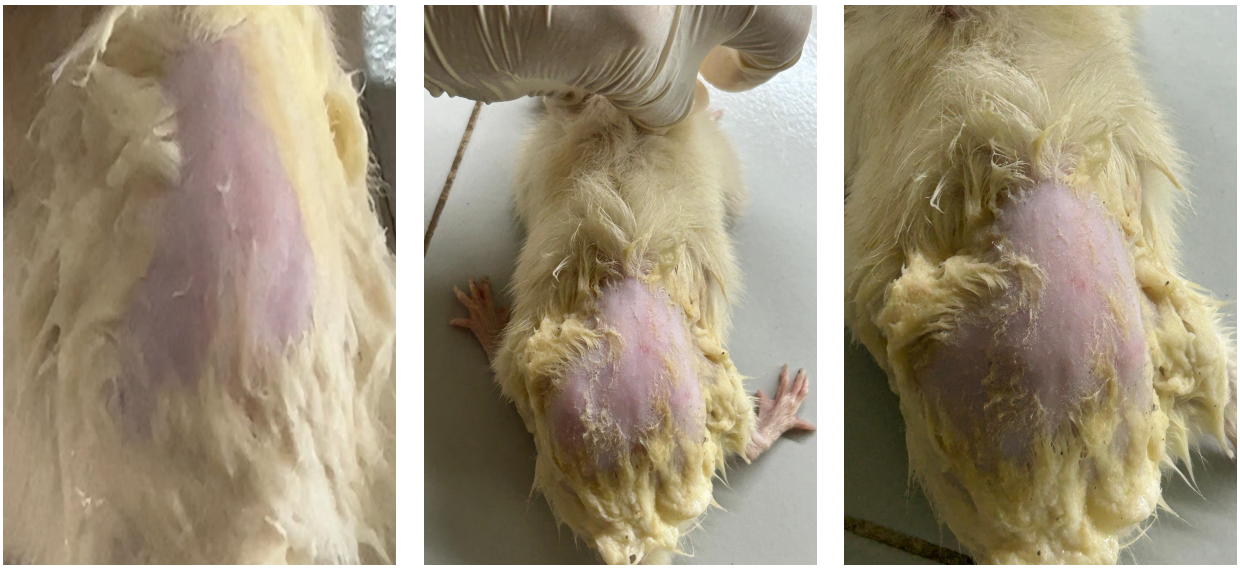
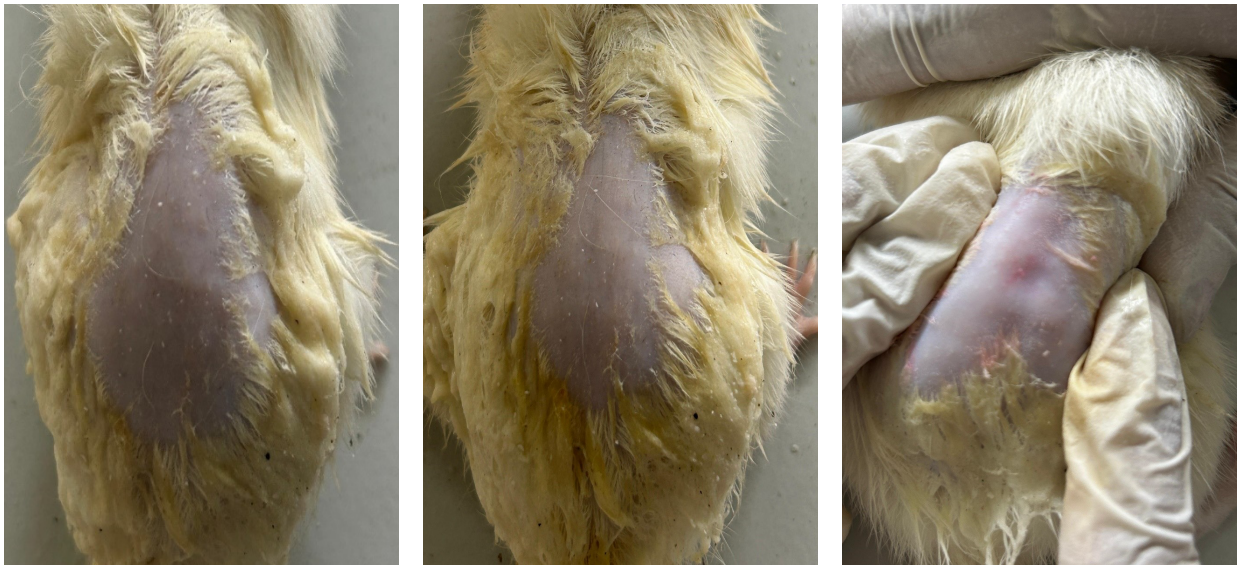


4(A) 4(B) 4(C)

Figure 4 Irritancy test on Positive control group at 0 minute **4(A),** 5 minutes **4(B)** and 15 minutes **4(C)** time intervals respectively



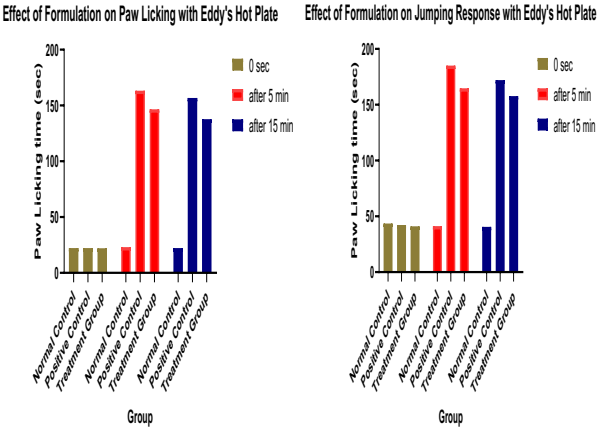
5(A) 5(B) 5(C)

Figure 5 Irritancy test on Treatment control group at 0 minute **5(A),** 5 minutes **5(B)** and 15 minutes **5(C)** time intervals respectively

In the normal control group, minimal variation in red intensity was observed over time (0 min: R=165; 5 min: R=191; 15 min: R=180), indicating no signs of skin irritation. Similarly, the positive control group (talcum powder) exhibited stable red values (0 min: R=174; 5 min: R=177; 15 min: R=173), confirming non-irritant behaviour. In contrast, the negative control group, treated with sodium lauryl sulphate (SLS), showed a substantial increase in red intensity at (0 min: R=182; 5 min: R=255; 15 min: R=208) reflecting a strong erythema response, confirming significant irritant potential. The treatment control group, which received the test (pre-treatment) formulation, demonstrated no appreciable increase in redness throughout the observation period. The red values remained low and stable (0 min: R=114; 5 min: R=115; 15 min: R=124), indicating that the formulation was non-irritant and well tolerated upon topical application. These findings validate the test formulation as a safer and more skin-friendly alternative for topical application.

Analgesic activity

Following topical application of the respective formulations, the analgesic effect was evaluated using paw licking latency and jumping response times on the Eddy's hot plate shown in **figure 6 and 7** respectively. Reaction



times were recorded at 0, 5, and 15 minutes to assess the onset and duration of analgesic activity.

Figure 6 Effect of paw licking and jumping with Eddy's hot plate

The normal control group showed consistently low latency times across all intervals, indicating the absence of any analgesic effect. In contrast, the positive control group (2% lidocaine gel) demonstrated a significant increase ($p < 0.05$) in response latency, confirming the validity of the model. Importantly, the treatment control group—which received the test (pre-treatment) powder formulation—also showed a statistically significant ($p < 0.05$) increase in both paw licking and jumping latency compared to the normal control. Although the analgesic effect of the test formulation was slightly lower than that of lidocaine, it remained consistently elevated throughout the observation period. This increase in latency times in the treatment group suggests effective pain threshold elevation, likely due to the synergistic action of the herbal constituents, including menthol and Arnica montana.

CONCLUSION

The current research presents a novel and scientifically optimized pre-treatment powder formulation intended to reduce pain and discomfort associated with hair removal techniques such as waxing and threading. The combination of Arnica montana and menthol provided effective herbal anesthetic action, while kaolin, rice powder, and talc contributed to improved water absorptivity and flow properties, ensuring both functional performance and ease of application. Comprehensive Preformulation studies confirmed the physical suitability of each component, and the use of a 3×2 full factorial design allowed precise optimization of key formulation variables. The selected batch demonstrated excellent flowability, desirable particle size, and water absorption capacity. Further, animal studies confirmed the analgesic efficacy of the formulation as well as its non-irritant nature, highlighting its safety and effectiveness for topical use. The optimized formulation offers a safe, cost-effective, and efficient pre-treatment option for hair removal. Its application prior to procedures like waxing or threading can reduce pain and irritation, improving overall user comfort. With proven analgesic and non-irritant properties, the formulation demonstrates strong potential for integration into routine dermatological and cosmetic practices.

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