



FORMULATION OF HERBAL HAIRGEL AND EVALUATION OF ITS ANTIDANDRUFF POTENTIAL

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1. ABSTRACT

Previous studies have indicated that dandruff affects over 50% of adult global population. It is a common condition that often leaves the scalp inflamed and itchy. Usually, individuals that have dandruff suffer with hair loss, reduced hair growth, thinning of hair and formation of skin flakes on the scalp, hair, eyebrows, beard and Mustache. The objective of this study was to conduct a phytochemical screening and evaluation of the so chosen medicinal herbs. The adverse effects of chemical medication have led to an increase in the popularity of traditional medicine. A major contributing element to dandruff is the fungus *Malassezia*. In the present study the plant materials such as amla, neem leaves, patha leaves, and tulsi are used in order to produce the required antidandruff action due to their potency in antioxidant activity, anti-inflammatory and antifungal property. A series of processes were followed in the preparation. It began with the procurement of the herbs, evaluation, preformulation studies, freeze drying and formulation. To the hair gel, a series of evaluation parameters were tested including *in vitro* and *in vivo* studies. Most of the drugs in the market have developed multi drug resistance to fungus hence it is necessary to develop a new class of antifungal drugs from natural sources (medicinal plants). In this study, we are using the following herbs like Amla (*Phyllanthus emblica L.*), Neem Leaves (*Azadirachta indica A. Juss.*), Tulsi (*Ocimum sanctum*), Patha leaves (*Cyclea peltata*) and Aloe vera (*Aloe barbadensis miller*). Studies have proved that drugs belonging to classes of secondary metabolites such as alkaloids, terpenoids, flavonoids, steroids, tannins and phenolic compounds are responsible for anti dandruff activity against a few species of the *malassezia* fungi (*M. globosa* and *M. restricta*).

KEYWORDS: Dandruff; Antidandruff; Antifungal; *Malassezia furfur*; Seborrheic dermatitis; Herbal treatment.

1. INTRODUCTION

Dandruff is a prevalent and non-contagious hair problem that can affect people of all ages. Medically, dandruff is defined as pityriasis simplex capitis, which refers to the shedding of dead skin from the scalp. Dandruff can manifest as either dry or greasy flakes. Dry dandruff appears silvery and white, while greasy flakes are pale yellowish and may have an unpleasant smell. There are historical names for dandruff that reflect its fungal causes, such as pityriasis simplex and pityriasis capitis (related to *Pityrosporum*) and *furfuracea* (related to *Malassezia furfur*). It emphasizes that dandruff is a common and potentially embarrassing disorder, affecting about 5% of the world keratinocytes, a type of skin cell, plays a significant role in the expression and generation of immunological reactions during the formation of dandruff.

To address the side effects of dandruff and seborrhea, there is an attempt to formulate and evaluate a Polyherbal anti-dandruff hair gel. The current treatment options available for seborrheic dermatitis are compounds that include Selenium sulfide, zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, and coal tar derivatives. However, these treatments have limitations, such as poor clinical efficacy or issues with compliance, and they often fail to prevent recurrence. As a result, there is an attempt to evaluate the anti-dandruff activity of herbs traditionally used by the tribes of the Wayanad district as an alternative approach. Selection of specific herbs for a polyherbal preparation aimed at addressing dandruff issues. The chosen herbs are Amla (*Phyllanthus emblica L.*), Neem Leaves (*Azadirachta indica A. Juss.*), Tulsi (*Ocimum sanctum*), Patha leaves (*Cyclea peltata*), and Aloe vera (*Aloe barbadensis miller*). These herbs have been traditionally used by the tribes of the Wayanad district and are known

to possess significant antifungal, anti-inflammatory, and antioxidant activities.

2. METHODOLOGY

A. Procurement of Herbs

Amla (*Phyllanthus emblica L.*)

In the month of March, the amla fruit was bought from the Kalpetta, Wayanad, Kerala, local market. In a nutshell, the amla fruit that had been gathered cleaned methodically under running water to get rid of any dust or other dirt that had stuck to them. The seeds found inside the amla fruit were extracted with care. One kilogramme of clean amla fruit was chopped into tiny pieces and dried in a hot air oven set to forty degrees Celsius for seventy-two hours. For later usage, the dry material was pulverised and kept in an airtight container.

Neem Leaves (*Azadirachta indica A. Juss*)

at March, the leaves of the neem plants were harvested at Wayanad, Kerala. In a nutshell, the gathered leaves were thoroughly cleaned under running water to get rid of any dirt or other material that had stuck to them. A hot air oven set at 40°C was used to dry one kilogramme of clean leaves over the course of a day. For later usage, the dry material was pulverised and kept in an airtight container.

Tulsi (*Ocimum sanctum*)

In the month of March, the aerial parts of tulsi were gathered from different locations in Wayanad, Kerala. In a nutshell, soil and other detritus that had attached itself to the collected plant pieces were rinsed out in a methodical manner under running water. A hot air oven set at 40°C was used to dry 1 kg of clean plant components over the course of 24 hours. For later usage, the dry material was pulverised and kept in an airtight container.

Patha leaves (*Cyclea peltata*)

In the month of March, the patha leaves were gathered from several locations throughout Wayanad, Kerala. In a nutshell, the gathered leaves were thoroughly cleaned under running water to get rid of any dirt or other material that had stuck to them. A hot air oven set at 40°C was used to dry one kilogramme of clean leaves over the course of a day. For later usage, the dry material was pulverised and kept in an airtight container.

Aloe vera (*Aloe barbadensis miller*)

In March, the ripe aloe vera leaves were harvested from Wayanad, Kerala. In a nutshell, the gathered leaves were

thoroughly cleaned under running water to get rid of any dirt or other material that had stuck to them. One kilogramme of clean, fresh leaves were divided into small pieces and ground in a mixer. Ultimately, the liquid was strained through a strainer after the solid was squeezed to extract the juice.

B. Extraction

The plant materials (amlam, neem leaves, patha leaves, and tulsi) which are cleaned, dried and finely powdered separately were used for extraction. The extraction is done by using the Soxhlet apparatus.

In the extraction processes, the round bottom flask is filled with one liter of purified water and 100 g of the powdered drug was measured and introduced to the thimble. Next, the thimble is inserted into the Soxhlet apparatus's extraction chamber. The condenser is also fitted to the Soxhlet apparatus. The RB Flask is warmed on a heating mantle making the liquid boil and its vapors, through the vapor tube, go to the condenser, and become concentrated, and the droplets that are left over land on the medication in the extraction chamber. Solvent is gradually added to the extraction chamber until the level rises over the siphon tube. At that point, the solvent runs through the siphon tube and back into the round-bottom flask, starting a new cycle. The plant extracts were collected and stored in the dark for further use. Its advantages over the general soaking method include low solvent flow rates, complete extraction, high efficiency, and a simple and clear design.

Freeze Drying

Freeze-drying has emerged as a crucial method for preserving the properties of herbal extracts. A laboratory-scale freeze dryer, specifically the Christ Alpha model from Germany, was employed for this purpose. The methodology involves the preparation of aqueous extracts/juice from various herbs, followed by a meticulous freeze-drying procedure. The frozen samples, stored at -80 °C, were transferred to the freeze dryer and subjected to -50 °C for 72 hours under a pressure below 0.110 mbar. The resulting dried product was finely powdered using a mortar and pestle, subsequently stored in an airtight container within a desiccator.

C. Formulation of Hair Gel

Using a gelling agent such as carbopol, the hair gel was made with herbal extracts. Composition of the formulated gel is given in Table 1.

Table 1: Formula for hair gel formulation.

Ingredients	Category	Official formula
Carbopol	Gelling Agent	1 g
Propylene glycol	Emollient & Viscosity modifier	15 g
Triethanolamine	Gel stabilizer by pH balancing	qs
Glycerin	Humectant	5 ml
Methyl paraben	Preservative	0.5 g
Chlorophyll Extract	Colouring Agent	5 ml

Freeze dried herbal extract	Antidandruff agent	qs
Tea tree oil	Perfume	qs
Purified water	Vehicle	qs 100g

Carbopol was added to the solvent mixture of propylene glycol, glycerin and water taken in a beaker and the resultant was agitated at 500 rpm for 2 hours using a mechanical stirrer. The mixture was slowly neutralized by adding triethanolamine drop by drop with constant stirring. Simultaneously the pH of the gel was measured with a pH meter. Triethanolamine was added until the gel achieved the required pH of 6.8. The required quantity of freeze dried herbal extracts and methyl paraben were dissolved in 25 ml of purified water

and this solution was added to the gel with gentle mixing. Sufficient quantity of chloroform extract as coloring agent and tea tree oil was added to the gel with gentle mixing. Finally the weight of the gel was adjusted up to 100 g with water. It was kept overnight for the polymer to swell to get the gel.

For the optimization of formulations, six sets of hair gel were prepared using the various concentrations of herbal extracts and the composition is given in Table 2.

Table 2: Composition of Formulations.

Sl No	Formulation Code	Quantity of Freeze Dried Herbal Extract Incorporated in Hair Gel (%)					
		Aloe vera	Neem	Patha	Tulsi	Amla	TOTAL (%)
1	HGF 1	1.25	1.25	2.50	2.50	1.25	8.75
2	HGF 2	1.25	1.25	5.00	2.50	1.25	11.25
3	HGF 3	1.25	2.50	2.50	2.50	1.25	10.00
4	HGF 4	1.25	2.50	5.00	2.50	1.25	12.50
5	HGF 5	1.25	5.00	5.00	2.50	1.25	15.00
6	HGF 6	1.25	5.00	2.50	2.50	1.25	12.50

D. Physico-mechanical Characterization

The formulation's content has a big impact on a gel's mechanical and physical characteristics. Each of the created hair gels underwent independent examination of the physico-mechanical parameters described below.

a. Physical appearance

We used both our hands and our eyes to analyse each generated gel's colour, scent, smoothness, and general look. The compositions' tactility and visual attractiveness were assessed to ascertain their homogeneity. The humectant quality of the gel was evaluated and assessed based on its colour, pearl essence, and roughness. Its slippery nature and the amount of residue left behind after applying a set quantity of gel were also investigated.

b. Homogeneity

To verify homogeneity, the generated gel was visually examined after it had been placed in the container. We looked at its appearance, aggregate content, and flocculates.

a. Determination of pH: Standardized solutions of buffers were used to calibrate the pH meter. In a beaker, 0.5 g of the gel was weighed, dissolved in 50 ml of pure water, and the pH was recorded.

d. Viscosity: Viscosity of the product helps to study flow properties and thixotropic behavior of the product. These studies were conducted using a Brookfield viscometer, a widely accepted instrument for evaluating the rheological properties of substances. The readings were

taken at 30 and 60 rpm to capture a range of rotational speeds.

e. Washability

The hair gel formulation is regularly evaluated for water washability by applying it to a glass slide.

f. Extrudability

Fillable tubes with the prepared hair gel composition are used. The material is extruded by pressing the tube, and the formulation's extrudability is verified.

g. Spreadability

The 2g of weighed gel was positioned between two glass slides. On the slides, 500g of weight was applied. For five minutes, the weight was applied for the predetermined amount of time. The spread circle's diameter was then measured at various locations after the weight was removed. Spreadability was computed via a mathematical procedure.

$$S = M \cdot L / T$$

Where, S = Spreadability,

M = Weight placed on the slide,

L = Diameter of circle in cm,

T = Time in seconds.

E. Evaluation of Anti-dandruff Potential

The *Malassezia furfur* and *Candida albicans* strains used in the investigation of their antifungal activity were taken from the microbiology lab of Dr. Moopen's Medical College Wayanad. In 100 millilitres of distilled water, 20 grammes of potato infusion, 2 grammes of dextrose, and 1.5 grammes of agar were dissolved to create the potato dextrose agar medium. The dissolved medium

was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. While still molten, the autoclaved medium was well combined and then transferred onto 100 mm petri plates (25–30 ml/plate).

Differently prepared hair gels were put to petri plates that contained 20ml of potato dextrose agar medium. The plates were seeded with a 72-hour culture of two fungus strains, *Candida albicans* and *M. furfur*. After that, the plates were incubated for 72 hours at 28°C. The diameter of the inhibition zone that developed around the wells served as an indicator of the anti-fungal action. A positive control was employed, namely amphotericin B. Software called Graph Pad Prism 6.0 (USA) was used to calculate the numbers.

F. Evaluation of Antioxidant Potential

The total antioxidant capacities of the prepared hair gels were determined by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. 0.5g of hair gel was added to 50ml of ethanol and mixed well in a separating funnel. In a nutshell, 100 millilitres of methanol were used to dissolve 4 milligrammes of DPPH, and the mixture was then homogenised in an ultrasonic bath for half an hour. Subsequently, 1.6 millilitres of the ethanolic solution extracted from the separating funnel was thoroughly combined with 2.4 millilitres of DPPH solution. The UV-Visible spectrophotometer was then used to measure the solution's absorbance at 517 nm. The following formula was used to determine the scavenging activity.

$$\% \text{ Scavenging activity} = \frac{(A_{517 \text{ control}} - A_{517 \text{ sample}})}{(A_{517 \text{ control}})} \times 100$$

4ml of DPPH solution is taken as blank, 2.4ml DPPH solution mixed with 1.4ml ethanol was taken as control. Ascorbic acid (100µg/ml) solution was used as standard.

Study Protocol

Protocol No.	: DMCP/AE/12/2023
CPCSEA Reg. No.	: CBLRC/IAEC/16/01-2023 (dated on 05/08/23)
Study Objective	: To compare the skin irritation property of formulated antidandruff hair gel with standard irritant.
Study Method	: Draize score method
Sample Size	: 24 healthy albino rats of either sex weighing about 150-200 g to be divided in to four groups (n = 6). A set of six rats have to be used in the study for each formulation.
Negative Control	: 0.2 ml normal saline solution (0.9% w/v Sodium Chloride solution)
Positive Control (Standard Irritant)	: 0.2 ml 0.8% v/v aqueous solution of formaldehyde
Placebo	: 0.2 g placebo gel without any herbal extract
Test	: 0.2 g of optimized formulation (HGF3)
Study Period	: 72 h
Score Scale	: Erythema scale: 0-none, 1-slight, 2-well defined, 3-moderate and 4-scar formation. Edema scale: 0-none, 1-slight, 2-well defined, 3-moderate and 4-severe
Irritation Parameters	: Primary dermal Irritation (PDI) score and Primary Dermal Irritation Index (PDII)
Ethical Consideration	: The study was carried out as per Laboratory Animal Science Association (LASA) Good Practice Guidelines.

G. Evaluation of Anti-inflammatory Activity

By using human red blood cells obtained from the blood bank of Dr. Moopen's Medical College, Wayanad, an in vitro anti-inflammatory research was conducted on the freeze-dried extracts using the Human Red Blood Cell (HRBC) membrane stabilization test.

Blood suspension (10% v/v) was made using regular saline. One milliliter of specially made hair gel and one milliliter of 10% RBC solution make up the reaction combination... In this case, normal saline was introduced to the control test tube in place of the test sample. 200 µg/mL of diclofenac was the typical medication. Every reaction mixture was incubated for 30 minutes at 56°C in a water bath. Test tubes were centrifuged for five minutes at 2500 rpm after being cooled under running tap water at the conclusion of the incubation period. The coloured supernatants' absorbance was measured at 560 nm, and the percentage of cell membrane lysis inhibition was computed. For every test sample, the experiment was run in triplicate. The formula was used to determine the percentage of membrane stabilization activity. [Chioma AA, et.al].

H. PRIMARY SKIN IRRITATION STUDY

Dermatitis is a permanent inflammatory skin condition that develops following the application of a test substance. The purpose of the study was to determine the degree of irritation that a specifically created antidandruff hair gel would cause when administered topically to wister albino rats. The results of this study can be used to assess the possible risk that could arise from exposing the skin, hair, or scalp to the test chemical. It was developed in order to meet the demands of the OECD guidelines for acute skin irritation chemical testing.

7.2.2. METHODOLOGY

The initial skin irritation test was conducted on twenty-four healthy albino rats, weighing between 150 and 200 grams, of both sexes. The animals were kept with unlimited access to water and regular animal feed. The animals were housed in a typical laboratory setting. One day before the trial began, the rats' dorsal locations had around 10 percent of their total body surface area reduced. For a full day, the animals in their pristine cages were not disturbed. Four groups, each with 6 rats, were formed from the rats. Group IV (Test Group I) received 0.2g of formulated anti dandruff hair gel (HGF3), Group III (Placebo Group) received 0.2g of placebo gel without any herbal extract, and Group I (Negative Control Group) received 0.2ml of normal saline solution. Group II (Positive Control Group) received 0.2ml of 0.8% v/v aqueous solution of formaldehyde as a standard irritant. The skin of the tested area was examined for dermal reactions of erythema/edema after the test article was applied for 24, 48, and 72 hours. The skin was then scored using the Draize scoring criteria (erythema scale: 0-none; 1 very slight, barely perceptible; 2-well defined; 3-moderate; 4-beet redness and scar formation). Edema scale: 0-none; 1-very mild, hardly noticeable; 2-well defined, by rising the boundaries of the affected region; 3-moderate (increased by about 1mm); 4-severe, by raising more than 1mm and going beyond the exposed area). The difference between the sum of the erythema and edema scores at a given time divided by the number of observations for the treated sites and the sum of the erythema and edema scores at a given time divided by

the number of observations for the control sites was used to calculate the Primary Dermal Irritation (PDI) score. By "adding the PDI scores for 24, 48, and 72 h scoring intervals divided by the number of scoring intervals," the Primary Dermal Irritation Index (PDII) was determined.

I. STABILITY STUDY

Stability testing is used to establish recommended storage conditions, retest intervals, and shelf lives by supplying evidence on how the quality of a drug substance or drug product varies over time under the influence of various environmental factors like light and humidity.

The stability studies in this paper were conducted in accordance with ICH recommendations. For a duration of two months, the glass container containing the optimized hair gel formulation (HGF3) was placed in a humidity chamber that was kept at 30 ± 2 °C / 65 ± 5 % RH and 40 ± 2 °C / 75 ± 5 % RH. Samples were examined and noted for any alterations in their physical attributes and anti-dandruff potential at the conclusion of the studies.

1. RESULTS

A. Formulation of hair gel

Aqueous based hairgel was prepared with optimized base cream formulation. In the present study, a total of six hairgel formulations were prepared using freeze dried herbal extracts.



B. Physico-mechanical Characterization

The composition of formulation has a significant effect on its physical and mechanical characteristics of cream. The prepared creams were individually analyzed by the various physico-mechanical characteristics.

Physical appearance

All the formulated gels were inspected for colour, odour, appearance, flexibility, homogeneity and smoothness through observation and touch. All the prepared gels

were found to be smooth, soft and homogeneous. All formulations produce a uniform distribution of herbal extracts in gel. Emolliency, slipperiness and amount of residue left after the application of fixed amounts of gel was found good. It was found that there is no change in organoleptic properties of gel.

However, the formulations HGF4 and HGF5 were found to be unstable and inelegant due to their hardness and dryness. Since they were aesthetically not acceptable as cosmetic formulations, they were eliminated from the further evaluations.

ii. pH

The pH of the prepared gels were measured using a calibrated pH meter and values of all the formulated creams are tabulated in Table 3,

Table 3: pH of the prepared herbal hairgels.

F.Code	pH of Cream
HGF1	5.67
HGF2	6.21
HGF3	6.76
HGF6	6.53

The pH of all the formulated hair gels was found to be in between 5.6-6.8 which is good for skin pH. All the formulations were shown pH nearer to scalp and hair required.

C. Evaluation of Antifungal Potential

Table 5. SD± Means of zone of inhibition obtained by sample Formulation against *M.furfur* and *C.albicans*.

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm)				
			HGF-1	HGF-2	HGF-3	HGF-6	PC
1.	<i>M. furfur</i>	Formulated hair gel	17.25	20.25	22.5	15.25	30.5
			± 0.70	± 0.35	± 0.3	± 0.3	± 0.707
2.	<i>C. albicans</i>	Formulated hair gel	10.5	12.5	15.25	9.25	17.5
			± 0.7071	± 0.70	± 0.35	± 0.35	± 0.70

n = 6, SD – Standard Deviation, PC -Positive Control, *Significance - p< 0.05

iii. Viscosity

The viscosity of the prepared gels was measured using a Brookfield viscometer. The reading was taken at 30 and 60 rpm using spindle no.4.for each gel and reported on Table 4.

Table 4: Viscosity of the prepared herbal hair gels.

F.Code	Viscosity at 30 rpm (cps)	Viscosity at 60 rpm (cps)
HGF1	3724	3583
HGF2	3542	3308
HGF3	3651	3427
HGF6	3238	3124

The viscosity of gels were found to be in between 3000 - 4000 cps which indicates that the formulated hair gels are easily spreadable by small amounts of shear. HGF1 and HGF3 showed good spreadable property than other formulations.

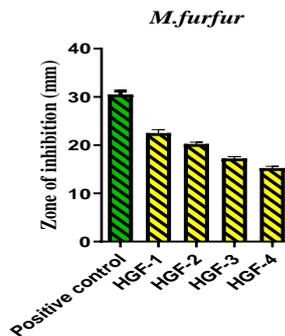
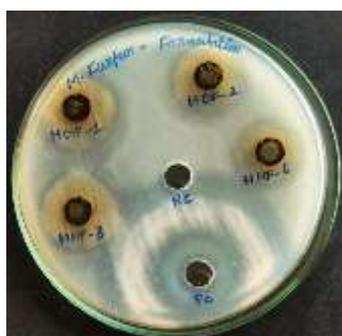


Fig : 11 Effect of Formulated hairgels against *M. furfur*.

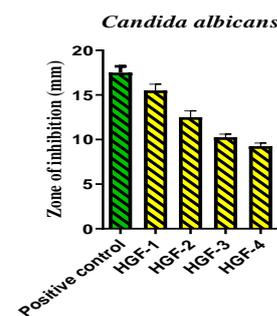
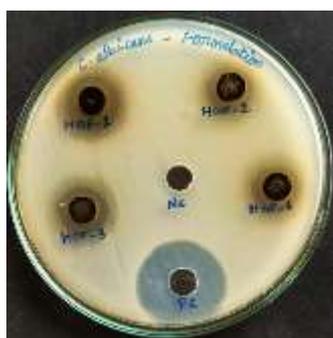


Fig : 12 Effect of sample Formulation against *C. albicans*.

D. Evaluation of Antioxidant Potential

The total antioxidant capacities of the formulated hair gels were determined by DPPH free radical scavenging activity in which the degree of discoloration of the DPPH

solution is an indication of extend of antioxidant property of the compound. Clear DPPH solution is taken as blank and ascorbic acid solution was used as the reference standard.

Table 6: Antioxidant activity of formulated hairgels in terms of percentage scavenging activity.

Sl. No.	Formulation	% Scavenging Activity
1	HGF1	62.73 ± 2.58
2	HGF2	68.81 ± 2.62
3	HGF3	75.09 ± 2.14
4	HGF6	70.34 ± 2.22
5	Standard (Ascorbic acid solution 100µg/ml)	97.06± 1.04

E. Evaluation of Anti-inflammatory Activity

In vitro anti-inflammatory study was carried out on the formulated herbal hair gels by Human Red Blood Cell

(HRBC) membrane stabilization test using the human blood collected from the blood bank of Dr. Moopen's Medical College, Wayanad.

Table 7: Effect of formulated hairgels on cell membrane protection.

Sl. No.	Formulation	% Protection
1	HGF1	52.13 ± 0.95
2	HGF2	58.49 ± 1.27
3	HGF3	62.58 ± 1.16
4	HGF6	61.20± 1.56
5	Standard (Diclofenac Sodium 50µg/ml)	71. 62± 1.32

F. OPTIMIZATION OF FORMULATION

G. The formulation HGF3 was chosen as the ideal formulation based on its physico-mechanical properties as well as its antindruff, antioxidant, and anti-inflammatory properties.

albino rats in order to rule out any chance of potential dermal irritation.

H. Skin Irritancy Study

Using the procedure outlined by Draize et al. (1944), the skin irritancy study was carried out for the optimised hair gel formulation and commercialised hair gel on healthy

Table 5 displays the skin irritation scores (erythema and edoema) of the optimised formulation (HGF3) and marketed hairgel, as well as the Primary Dermal Irritation Score (PDI) and Primary Dermal Irritation Index (PDII) that were computed during the skin irritation test.

Table 5.40. Skin irritation scores assigned during skin irritation test of formulated antidandruff hairgel (HGF3)

Group	Rat No.	24 h		48 h		72 h	
		Eryt	Edem	Eryt	Edem	Eryt	Edem
Group III Placebo (Normal saline solution)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	0	0
	PDI	0		0		0	
	PDII	0					
Group II Positive Control (Formalin Solution)	1	1	1	2	1	3	2
	2	2	2	2	3	2	3
	3	2	1	1	2	3	3
	4	1	2	2	2	3	3
	5	2	2	1	1	3	2
	6	2	2	2	3	2	3
	Mean	1.667	1.667	1.667	2.0	2.667	2.667
	SD	± 0.577	± 0.577	± 0.577	± 1.0	± 0.577	± 0.577
	PDI	3.334		3.667		5.334	

	PDII	4.112					
Group III Placebo (placebo gel without any herbal extract)	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	0	0
	PDI	0		0		0	
	PDII	0					
Group IV Test I (Optimized Formulation: HGF3)	1	0	0	0	0	0	0
	2	1	0	0	0	0	0
	3	0	0	0	0	0	0
	4	1	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	Mean	0.333	0	0	0	0	0
	SD	\pm 0.577	0	0	0	0	0
	PDI	0.33		0		0	
	PDII	0.11					

Eryt: Erythema (0-none, 1-slight, 2-well defined, 3-moderate and 4-scar formation)

Edem: Edema (0-none, 1-slight, 2 - well defined, 3 - moderate and 4 - severe)

PDI: Primary Dermal Irritation Score.

PDII: Primary Dermal Irritation Index.

For the standard irritant (formalin solution), the PDII value was 4.112 in the trial, while the value for the negative control was 0. and it was discovered that the value for the hairgel formulation (HGF3) was 0.11.

Substances that cause skin irritation scores of 2 or lower are regarded as negative (no skin irritation), according to Draize et al. (1944). Erythema and edoema skin irritation ratings of fewer than two were found in the skin irritation research conducted on the optimised hairgel formulation. Because of this, the designed hairgel was safe to use and did not cause skin irritation (Ubaidulla et al., 2007; Shinde AJ et al., 2010). Figure 5 displays the photos that were taken.

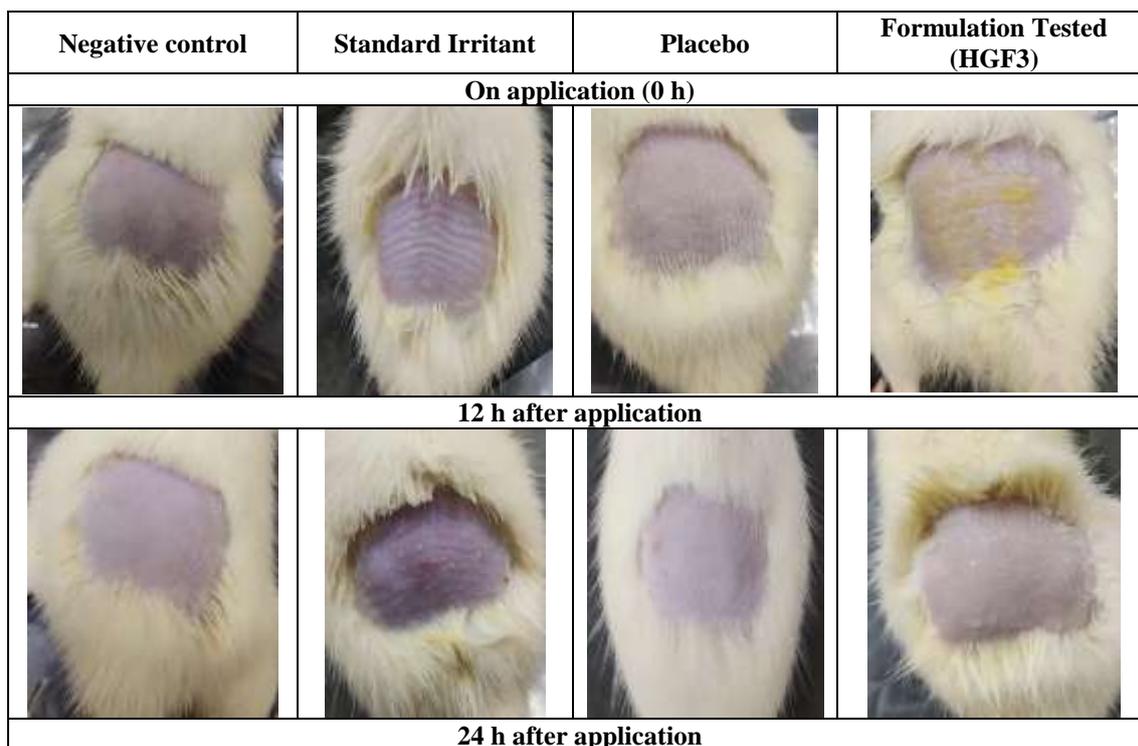




Figure 5: Skin irritation study performed on albino rats.

I. STABILITY STUDY

For two months, at 30 ± 2 °C / $65 \pm 5\%$ RH and 40 ± 2 °C / $75 \pm 5\%$ RH, stability investigations were conducted on the optimised formulation (HGF3) and the commercial formulation in a stability chamber. At 0, 30, and 60 days, six samples of the tested formulation were taken out at regular intervals, and their physical features, physico-mechanical traits, and antidandruff potential were examined.

The physical-mechanical properties and antidandruff potential did not change much even after 60 days of storage, according to the data.

CONCLUSION

Based on the results, it was determined that the hairgel formulation has strong anti-inflammatory, anti-oxidant, and antifungal characteristics, making it an effective anti-dandruff agent. Both in vitro and in vivo experiments were conducted on the developed hairgel mixture. Rats were used in the formulation's skin irritation test, and the results showed that the formulation was safe to use. A test was conducted on each gel to determine its effectiveness the gels were subjected to a test where in the effect of the developed gel was checked against the species (1) *M.furfur* and (2) *C.albicans* - this test concluded that the formulations showed positive results for the required anti-fungal property. Using ascorbic acid as the reference to verify the scavenging activity of the formulations, the DPPH method was utilised to assess the antioxidant property of the gels. The human red blood cell (HRBC) was used to test the anti-inflammatory property. The HGF3 was chosen as the ideal formulation after comparison using the aforementioned tests, scavenging activity, and evaluation. A test was conducted on each gel to determine its effectiveness. The samples were taken at

various points of time after storage, namely 0, 30, and 60 days. The formulation didn't change significantly in terms of its physicochemical characteristics, and it stayed stable. The HGF3 was therefore shown to exhibit the required qualities.

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