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ORIGINAL ARTICLE

Formulation and Evaluation of Green Tea Gel for the Treatment of Vitiligo

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ABSTRACT
Vitiligo, the most prevalent depigmentation disorder, is characterized by the emergence of white patches on the skin. The root cause of vitiligo is primarily attributed to the autoimmune destruction of melanocytes. Notably, oxidative stress plays a pivotal role in this process, leading to molecular and organelle dysfunction within melanocytes. It also contributes to the exposure of melanocyte-specific antigens and eventual melanocyte cell death, thereby significantly influencing the progression of vitiligo.In recent years; there has been a growing interest in the potential therapeutic effects of natural products in the context of various skin diseases. This review is specifically centered on exploring the impacts and underlying mechanisms of natural compounds in models related to vitiligo. Some natural compounds, including flavonoids, phenols, catenin, and coumarins, have demonstrated protective properties for melanocytes, effectively curbing depigmentation. Among these natural compounds, epigallocatechin-3-gallate (EGCG) stands out as a major polyphenolic component found in green tea (Camellia sinensis). EGCG is renowned for its antioxidant, anticancer, and anti-inflammatory attributes. Research has unveiled its capacity to shield retinal pigment epithelium (RPE) from oxidative stress-induced cell damage. Moreover, Epigallocatechin gallate (EGCG) has the potential to delay the onset of vitiligo, mitigate its aggressiveness, and reduce the extent of depigmented areas. Application of green tea on depigmented skin sites has shown promise in enhancing melanin density, a crucial prerequisite in the treatment of vitiligo.
Keywords; *Green Tea Polyphenols, Vitiligo, Melanocytes, Melanin, Depigmentation, Autoimmune Adapalene.*

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INTRODUCTION

Vitiligo, a prevalent dermatological condition marked by depigmentation, is believed to impact a range of 0.5% to 2% of the world's population [1]. In this disorder, melanocytes, the cells responsible for determining skin color, are specifically depleted, resulting in the formation of white, non-scaly patches. These achromic patches and macules, characterized by the absence of pigmentation in the epidermis, represent a multifaceted expression of vitiligo [2].

Three primary factors contribute to the development of this condition. A significant factor involves a diminished reaction to allergens upon skin contact, an anomaly observed in the hypopigmented epidermis, akin to observations in hypopigmented rats [3]. Intriguingly, individuals with albinism, who share white skin characteristics with vitiligo patients, display a heightened vulnerability to skin carcinoma. In contrast, those with vitiligo do not develop non-melanoma skin cancer. Recent progress in comprehending the root causes of vitiligo has firmly classified it as an immunological disorder [4].

The precise origin of vitiligo remains elusive, frequently linked to autoimmune disorders and shaped by a combination of genetic and environmental elements. It presents intricate hereditary characteristics, potentially associated with melanin synthesis, autoantibodies, and reactions to oxidative stress. Vitiligo, the primary cause of skin depigmentation, usually manifests during the second and third decades of life, with onset variability between genders. It impacts a global range of 0.1% to 2% of people, regardless of

their racial background, affecting individuals from childhood through adulthood [5].
Treatment for vitiligo is often regarded as elective due to its non-threatening nature and its main influence on one's physical appearance. Nevertheless, if extensive vitiligo or its effect on your emotional health raises concerns, your healthcare provider can help you explore potential treatment choices [6]. These strategies aim to attain a uniform skin tone by either reinstating pigment (repigmentation) or eliminating existing pigment (depigmentation). Typical methods for addressing vitiligo include pharmaceutical treatments, phototherapy, depigmentation procedures, surgical interventions, and counseling [7].
Epigallocatechin-3-gallate (EGCG) stands out as a primary chemical compound found in green tea, a significant component of traditional Chinese medicine. Green tea is renowned for its anti-inflammatory, antioxidant, and immune-regulating characteristics. Yet, the impact of EGCG on vitiligo remains uncharted territory [8]. The study investigated the influence of EGCG in a mouse model of vitiligo induced by monobenzone. EGCG was observed to prolong the onset of depigmentation, lower the occurrence of depigmented patches, and reduce the size of depigmented areas. Examination of the depigmented skin treated with EGCG using reflectance confocal microscopy revealed an increased presence of epidermal melanocytes, while histological examination indicated a decrease in the accumulation of CD8(+) T cells in the perilesional region [9]. Although often downplayed as a cosmetic issue, vitiligo can yield profound psychological repercussions and substantially disrupt everyday life. The therapeutic potential of EGCG in addressing vitiligo underscores EGCG as the primary and pivotal polyphenol in green tea. Various health-enhancing attributes of EGCG, such as its antioxidant and anti-inflammatory properties, have been well-documented [10]. The review titled "Herbal Compounds for Vitiligo Treatment," suggests that green tea polyphenols could have potential utility in addressing vitiligo by mitigating oxidative stress within the melanocyte unit [11].

MATERIAL AND METHODS

Dried green tea leaves are sourced from Nashik's local market, while other chemicals such as orange oil, Carbopol 940, Glycerin, Triethanolamine, Methylparaben, and Propylparaben are procured from Sudarshan Chemical Agency in Mumbai.

Method of preparation

The steps carried out in the preparation of green tea gel were as follows,

Preparation Sample: The collected samples of green tea leaves were cleaned of dirt adhering to the leaves using running water and then dried by air drying. After drying the sample is then powdered.

Preparation of Green tea extract: The powdered substance was subjected to maceration in 250 ml of distilled water for 4 hours, after which the resulting extract was filtered through cotton wool. Subsequently, the filtrate underwent drying and concentration.

Preparation of Gel base: Carbopol 940 was gently dissolved with continuous stirring in 60 mL of demineralized water for 1 hour to prevent clumping. Subsequently, Glycerin and triethanolamine were separately dissolved in 10 mL of demineralized water each, with stirring for 10 minutes. These solutions of Glycerin and triethanolamine were then combined with the Carbopol solution, and the pH was adjusted to 7.4 by stirring the mixture for another 10 minutes. Following this, the propylene glycol solution was introduced while stirring for an additional 10 minutes, until a clear and uniform gel base was achieved.

Preparation of gel formulation: Three topical gel formulations were created utilizing Aqueous Green Tea Extract (AGTE) and orange oil following the drug formulation manual. Formulations denoted as F1 to F3 were developed using a gel base consisting of 2% carbopol 940. The specific formulation compositions are documented in Table I.

Table 1: Formulation of gel

Ingredient	Role	F1	F2	F3
Aqueous Green tea extract (AGTE)	Helps in repigmentation	2.5 gm	3.0gm	3.5 gm
Orange oil	Provides Nourishment and perfume	1.5 ml	1.5 ml	1.5 ml
Carbapol940	Gelling agent	0.2gm	0.4gm	0.4gm
Glycerin	Moisturizers	5 ml	8 ml	10 ml
Propylene glycol	Gelling agent	5ml	5ml	5ml
Triethanolamine	PH adjuster	0.1 ml	0.1 ml	0.2 ml
Methylparaben	Preservative	0.2gm	0.2gm	0.2gm
Propylparaben	Preservative	0.1gm	0.1gm	0.1gm
Distilled water	Solvent	Upto100 ml	Upto100 ml	Upto100 ml

Method of preparation

Mechanical Homogenization method

Precisely measured Carbopol 940 was placed into a beaker and dispersed in 50 ml of distilled water. The beaker was set aside for 24 hours to allow Carbopol 940 to swell. Following this, continuous stirring was carried out using a mechanical/lab stirrer at 1200 rpm for 30 minutes. Subsequently, glycerin, propylene glycol, and orange oil were weighed and introduced into the mixture of propyl paraben and methyl paraben, ensuring thorough mixing. A total of 3.5 ml of green tea extract was then gradually added to the mixture while stirring consistently. Finally, the volume was adjusted to 100 ml by adding the remaining distilled water, and triethanolamine was incrementally added to the formulation.

MATERIAL AND METHOD

Evaluation of gel:

Physical evaluation

The physical evaluation like color, and odor, was observed visually. This is confirmed by the visual appearance of the touch.

Fig 1: Formulation

Appearance and Homogeneity

Physical appearance and homogeneity of the prepared gels were evaluated by visual perception.

Determination of pH

Place 1 gram of the sample into a beaker and pour 100 ml of distilled water over it. Thoroughly stir the mixture until the entire gel has dissolved in the water. Subsequently, immerse the calibrated electrode into the sample mixture using a pH meter.

Viscosity

The gel's viscosity was assessed by employing a Brookfield viscometer (model LVDV-E, S-62) at a temperature of 25°C, with the viscometer spindle rotating at a speed of 12 rpm.

Spreadability

Two sets of glass slides with standard dimensions were utilized. The green tea extractgel formulation was applied to one of the slides. The other slide was then positioned atop the gel, effectively sandwiching the gel between the two slides, covering an area measuring 7.5 cm along the slides. A weight of 100 grams was gently placed on the upper slide, ensuring even pressure to create a thin layer of the gel. Subsequently, the weight was removed, and any excess gel adhering to the slides was carefully removed.

The two slides were securely fastened in position on a stand, ensuring minimal disruption, allowing only the upper slide to slide freely due to the gravitational force exerted by a weight attached to it. A 20-gram weight was cautiously attached to the upper slide. The time required for the upper slide to traverse the 7.5 cm distance and separate from the lower slide under the influence of the weight was recorded. This experiment was conducted three times, and the average time was calculated for analysis.

Spreadability was calculated by using the following formula:

$S = m \times l/t$

where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time is taken in sec.

Skin irritation study

Ten male and female volunteers, who were in good health, were chosen for skin irritation assessments. A 100 mg quantity of gel was administered to a 2 cm section of the inner surface of the upper arm and then covered with a cotton bandage for 6 hours. Following these 6 hours, the application sites were cleansed with acetone and evaluations were conducted using the Draize scale.

No irritation: 0; Slight irritation: 1; Irritation: 2

Stability studies of topical green tea extract gel formulation

The primary aim of stability testing is to provide evidence regarding how the quality of the drug product

changes over time due to temperature and humidity influences. For the stability study of the green tea extract gel formulation, we followed the guidelines set by the ICH (International Council for Harmonization). This study was conducted over 6 months using a stability chamber.

The selected green tea extract gel formulation, which included 2% of both CHME and VNME, was placed in a humidity chamber (floor-standing model with three units, each equipped with individual humidity and temperature control) measuring 300 X 300 X 300 mm. The conditions in the chamber were maintained at 25°C ± 2°C/60% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% RH, and 40°C ± 2°C/75% RH ± 5% RH. Samples were retrieved at specific intervals, including the initial, first, second, third, and sixth months. These samples were then assessed for any alterations in color, odor, uniformity, pH levels, viscosity, net content, microbial contamination, and sterility using standardized testing procedures.

Retrospective study for Vitiligo

A retrospective analysis of medical records was conducted on vitiligo patients who were prescribed green tea gel between July 2023 and August 2023. All patients received instructions to apply green tea gel three times daily for 6 months, along with exposure to sunlight. The effectiveness of the treatment was evaluated by determining the percentage of patients achieving re-pigmentation of the target lesion equal to or greater than 75% after 24 weeks, classified as a "good response." Additionally, re-pigmentation of 50% or more was considered a "better response." The extent of re-pigmentation was assessed according to a predefined protocol and graded on a scale from 0 to 5. Safety was evaluated by calculating the percentage of patients who reported one or more adverse events at the end of 24 weeks.

RESULTS AND DISCUSSION

Generally, when considering various topical semi-solid preparations, gel formulation is favored. This preference stems from its extended duration on the skin, high viscosity, capacity to moisturize dry skin due to its occlusive attributes, heightened adhesion, reduced potential for irritation, independence from the water solubility of the active ingredient, ease of application, and improved release characteristics.

To create three distinct gel formulations denoted as F1, F2, and F3, we employed different concentrations (2.5%, 3.0%, and 3.5% w/w) of an aqueous green tea extract. In these formulations, Carbopol 940 served as the gelling agent, selected for its biodegradable nature, strong bioadhesive properties, biocompatibility, non-irritating qualities, and lack of absorption into the body.

Evaluation of gel:

We assessed the characteristics of three gel formulations labeled F1 to F12, which were created utilizing Carbopol 940 polymers. These evaluations encompassed parameters such as physical appearance, pH levels, viscosity, spreadability, net content, extrudability, and in vitro diffusion patterns. The outcomes of our investigation fell within the acceptable parameters established by the ICH guidelines, and you can find detailed information in Table No. 2.

Table 2: Evaluation parameters for topical green tea gel formulation made

Code	Conc (%)	pH	Viscosity (poise)	Spreadability g cm/sec	Net content % w/w	Extrudability	Physical appearance
F1	2.5	7.67	0.3867	32.25	99.7	Good	Yellowish, translucent, and smooth
F 2	3.0	7.38	0.3839	47.28	102	Excellent	Dark yellowish, smooth, translucent, homogenous
F 3	3.5	7.70	0.3870	51.63	103	Good	Dark yellowish, homogenous, smooth, translucent

The prepared gels exhibited uniformity and a pleasing visual appearance with consistent texture. Regarding pH levels, all formulations fell within the close range of neutral pH, ranging from 7.42 to 7.88, thereby causing no skin irritation. This finding is corroborated by the results of the skin irritation study.

Polymers were deliberately included in the designed topical formulations to ensure the rapid release of the drug and to achieve as well as maintain the drug concentration within the therapeutically effective range. An ideal viscosity range between 0.38 and 0.39 poise was reported for topical gel formulations utilizing Carbopol polymers.

Furthermore, the spreadability values indicated that the gel formulations were easy to spread. Among the gel formulations F1 to F3, more than 90% of the contents were found to be extrudable, signifying excellent extrudability. The only exception was F1, where 80% of the contents were extrudable, which still falls within the "good" range (>80% extrudability: good, >70% extrudability: fair).

Skin irritation test

We conducted a skin irritation evaluation of the green tea extract gel we prepared, and no signs of erythema or edema were detected for any of the formulations, even after a 10-day observation period. This outcome underscores the safety of the green tea extract gel formulation we developed.

Stability testing

To ensure the quality, safety, and effectiveness of the product over its shelf life, we conducted a stability study following the guidelines provided by the ICH, focusing on the F2 formulation due to its superior quality attributes. Throughout 0, 1, 2, 3, and 6 months of stability testing, no alterations were observed in terms of color, odor, uniformity, pH levels, viscosity, or net content of the topical green tea extractgel formulation for this specific formulation. These results unequivocally indicate that the formulated topical gel F2 maintains its stability over time. Furthermore, F2 displayed superior release characteristics, with a release rate of 98.4%, outperforming both F1 and F3 among the formulations.

Retrospective review for Vitiligo

The findings indicated that 11 patients, constituting 86.3% of the study participants, responded positively to the treatment regimen after three months: 2 patients experienced a repigmentation of 50–74%, 4 patients saw a repigmentation of 25–49%, 3 patients had a repigmentation of 1–24%, while 2 patients did not exhibit any repigmentation. Following the completion of six months of therapy, the outcomes were as follows: 1 patient achieved a repigmentation of 75–99%, 3 patients achieved 50–74% repigmentation, 5 patients observed 25–49% repigmentation, no patient experienced 1–24% repigmentation, and 1 patient showed no change in repigmentation. Remarkably, only 4 patients (9%) demonstrated a substantial 90% repigmentation (grade 6) at the study's conclusion. Furthermore, it's noteworthy that none of the patients reported any stinging, burning sensations, or pruritus during the initial week of treatment.

CONCLUSION

This gel formula, incorporating green tea extract, presents promise as a potential treatment for vitiligo. In summary, the gel infused with green tea extract exhibited favorable pH levels and spreadability attributes. It left no residue and was easily removable upon washing. Green Tea gel was deemed safe and moderately effective in treating vitiligo. However, to establish its efficacy and safety conclusively, it is advisable to conduct randomized controlled trials involving larger sample sizes. Consequently, Green Tea gel emerges as a viable alternative for treating vitiligo.

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