Hair Growth Promoting Effect Of Topical Poly Herbal Oil On Sprague Dawley Rats

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ABSTRACT
Hair loss is a common concern for both men and women, usually characterized by hair thinning, dandruff and extreme hair fall or shedding. Although synthetic remedies for hair loss are available but they often have appalling side effects and do not offer a permanent and long-lasting solution. The goal of the study was to formulate a herbal hair repair oil that would promote hair growth and repair while reducing hair loss. It was also aimed to examine and compare the effects of various herbal oils on hair growth in Sprague Dawley rats. For this purpose, a variety of herbs were selected including, the leaves of *Hibiscus rosa sinensis*, *Murraya koenigii*, *Melaleuca alternifolia*, *Monarda fistulosa* and *Salvia rosmarinus*. Castor oil, coconut oil, olive oil, almond oil, sesame oil, peppermint essential oil, and rosemary essential oil were among the other oils used in this investigation. The formulated repair hair oil was evaluated through physical, chemical, and hair growth tests by applying it topically to shaved albino rats. Primary skin irritation, antioxidant activity and hair length tests were performed. The results of the hair growth study were compared with locally purchased hair oils. Poly herbal hair repair oil demonstrated the best results among the tested samples in terms of increased hair length, efficacy, safety, promising physicochemical properties, antioxidant activity and hair growth promotion in animal models.

Keywords: Hair Growth, Sprague Dawley Rats, Anti-oxidant

INTRODUCTION:

The use of different hair oils has been well recognized worldwide since ancient times, however, it still remains obscure how they affect the hair and scalp. They are often readily available, inexpensive and affordable. People use different types of oils and new ones are emerging day by day. The majority of the hair oils primarily serve as emollients or conditioners but their distinct properties indicate that they have an even greater effect than that. This article focuses on different types of hair oils and the potential benefits they can provide to hair. Dermatologists ought to comprehend the effects of hair oils and their usage [1]. In Pakistan and all over the world, people use hair oil on their scalp because it is generally believed that it prevents hair loss, makes hair shiny and voluminous, and prevents greying for a long time. Hair oiling entails combing the hair and then applying oil from roots to tips, frequently with a hair braid before shampooing [2]. This belief is also used by the cosmetics industry, which has many over-the-counter personal hair care products. Dermatologists are often consulted for hair oil recommendations, thus it is necessary to understand common hair oils.[3]. Hair loss is a common health problem of a large population of both men and women accros the globe. Healthy hair is not just about appearance or aesthetics, it is an external manifestation of a person's general well-being. It's a reflection of how a human body works and herbs are really useful in fostering the luxuriant hair that one desires. Society puts pressure on both men and women to look a specific way because of the widespread interest in a person's body shape, hair, makeup and clothing [4]. Hair possesses significance for both sexual and social communication, as per studies. It has an immense impact on how individuals perceive one another in communal places and how the human body visually appeals. Hair, which is an appendage originating from the skin's ectoderm has defensive purposes and adds to the body's overall enticing qualities. Hair loss can be prompted by a variety of factors, including hormone fluctuations, vitamin deficiencies and genetics.

Thyroid disease is one medical problem that may culminate in hair loss or thinning and brittle hair [5]. Despite the fact that there is not a panacea for hair growth, studies have indicated that certain herbs may assist/encourage new hair growth or mitigate hair loss. However, it's crucial to remember that a substantial amount of research endeavor has been conducted on animals. Further research is required to demonstrate their efficacy in humans. In this journey through the arena of hair and herbs it's crucial to find the relationship between them and the best way to harness natural residences for wholesome hair boom and shine [6]. Herbs have been used as a natural treatment for ages. Their specific compounds provide a comprehensive method of hair care. Their advantages go beyond superficial enhancements; they focus on the underlying causes of a person's hair health. The intricately planned process of hair growth is frequently imperceptible until something interferes with it [7].To understand how herbs can interfere positively, let's examine the hair growth cycle and the elements that affect it. The three stages of the hair development cycle are anagen (growth), catagen (transitional) and telogen (resting). Since hair strands are at varying stages of growth, understanding this cycle helps explain why maintaining hair requires constant effort. Hair development is influenced by several factors, including nutrition, hormonal imbalances and genetic makeup. Herbs may contribute to promote healthy hair by addressing this interaction of internal and external causes [8] [9]. There are many benefits of using herbs to promote hair growth. They offer vital nutrients that hair needs, enhance blood flow to the scalp, and have anti-inflammatory qualities. Adopting herbs means accepting the notion that they can be utilized in hair care medicine and to alleviate the body [10]. This article will discuss different kinds of hair oils used in Pakistan along with their chemical makeup, potential effects and applications. Here, it is made clear that there is a dearth of scientific research on these subjects and that inflated claims on their impacts are frequently made without sufficient support or data from peer-reviewed journals. Relevant summaries of the content for which substantiation was accessible are attempted. The formulation that has been generated through this study can now successfully accomplish the previously indicated characteristics as well as other goals and benefits. In the light of aforementioned context, the purpose of the study is to prepare and evaluate a herbal hair repair oil that is intended to encourage hair growth. Additionally, a comparative assessment has been performed between the newly developed herbal hair repair oil and commercially available hair care products in order to assess their relative efficacy after undertaking experiments on laboratory animals' i.e, Sprague Dawley rats.

MATERIALS AND METHODS

Composition of poly herbal hair repair oil for hair growth and scalp health

This innovative hair oil formula fosters overall hair health and vitality by leveraging the synergistic qualities of its natural ingredients to address common hair and scalp problems. This cutting-edge hair formula is intended to safeguard against dandruff and its associated illnesses, accelerate hair growth and lessen hair loss. A combination of natural active substances with well-established health benefits is included in the overall composition.

Key Ingredients:

1. **Rosemary leaves**: Known for promoting circulation in the scalp, rosemary helps stimulate hair follicles, encouraging healthy hair growth [11].
2. **Tea Tree leaves**: With its antifungal and antibacterial properties, tea tree oil combats dandruff and soothes scalp irritation, ensuring a healthy environment for hair growth [12].
3. **Monarda leaves**: This herb offers soothing effects and supports overall scalp wellness, contributing to a balanced environment for hair [13].
4. **Curry patta (Murraya koenigii)**: Rich in nutrients and antioxidants, curry patta nourishes hair follicles and helps prevent hair loss [14].
5. **Hibiscus flowers**: Renowned for their conditioning properties, hibiscus strengthens hair and adds a natural shine, while also providing a cooling effect [15].
6. **Methidana (Trigonella foenum)**: Known for its ability to stimulate hair growth and enhance hair texture, it nourishes the scalp and prevents dryness [16].

Carrier Oils:

- **Coconut oil**: Serves as the base oil, providing deep hydration and nourishment due to its antioxidant-rich composition. It penetrates the hair shaft effectively, reducing protein loss [17].
- **Olive oil**: Offers restorative properties, moisturizing the hair and providing relief from scalp allergies [18].
- **Castor oil**: Supports hair growth and enhances scalp relaxation, while its thick consistency helps lock in moisture [19].
- **Almond oil**: Rich in vitamins, almond oil hydrates, repairs, and strengthens both hair and scalp, improving overall health [7].
- **Rosemary essential oil**: Enhances blood circulation, alleviates irritation, and contributes to a soothing scalp experience [20].
- **Sesame oil**: It is rich in vitamins and minerals, which strengthen hair follicles, reducing breakage. It offers some protection against sun damage. It deeply hydrates the hair and scalp, preventing dryness and has anti-inflammatory properties [21].

- **Peppermint essential oil**: Promotes blood circulation to the scalp, soothes scalp irritation and reduces dandruff, encouraging hair growth [22].

Usage:
A small amount of the prepared poly herbal hair repair oil should be applied to the hair and scalp. Gently massage for 5-10 minutes to promote absorption and encourage blood circulation. For optimal effects, let it stay on for at least half an hour or overnight, and then rinse with a mild shampoo. This formula offers a solution for those looking to repair hair while promoting growth and keeping skin healthy.

Method of preparation for poly herbal hair oil
Herb cleaning and drying:
Cleaned the rosemary, tea tree, and monarda leaves thoroughly and removed any contaminants. Dried the herbs fully in a dry, dark environment.
Herb infusion:
Infused the cleaned and dried herbs in 200 ml of coconut oil. Then kept the mixture in a dry and dark area allowing it to steep for 4 weeks. After four weeks, strained the oil to eliminate the herb residue, retaining the infused coconut oil.
Preparation of other oils and herbs:
Fine powder was made from curry pata, hibiscus flowers, and methidana, after grinding. Mixed this mixture with 200 ml of olive oil, 100 ml of almond oil, and 100 ml of sesame oil in a heat-proof container.
Heating the oil mixture:
The oil and the herb mixture container was placed in a water bath and heated gradually over a low flame. To maintain the medicinal qualities of oils and herbs, heated for around four hours while keeping the temperature low.
Final mixture:
Once heated, blended the oil and herb mixture with infused coconut oil. Lastly, for maximal even distribution, we added 5 ml of castor oil, 7 ml of rosemary essential oil and peppermint essential oil to the mixture.
Storage:
Transferred the finished poly herbal hair oil into a sterilized, dark glass bottle to maintain its effectiveness and protected from light. Kept in a dry, cool area. This technique is beneficial to give excellent attributes of each component to offer a natural, efficient remedy for hair development and scalp health.

Animals
Sprague Dawley rats of either sex were used in all experiments. Animals were procured from the Pharmacology Section of the Medicinal Botanic Centre, PCSIR Labs Complex, Peshawar, Pakistan. The animals were maintained in standard laboratory conditions (25°C and light/dark cycles i.e. 12/12h) and were fed with standard food and water *ad libitum*. The experimental protocols were approved by the ethical committee of the PCSIR Labs Complex, Peshawar, Peshawar, Pakistan.

Primary skin irritancy test
In order to evaluate the irritation effect of the prepared poly herbal hair repair oil formulation, the skin area of approximately 2cm^2 from the back of the animals was shaved. The herbal hair oil formulation (approx.1ml) was applied to the shaved area of intact skin of each rat. Observations were focused on the development of erythema (redness of the skin) and edema (swelling) for 72 hours after the application of test samples according to the Draize scoring system [23-25].
Hair growth activity
Five groups of six rats each were formed. Hair was shaved off from approx. 2 cm^2 area on the dorsal portion of the body of each rat and cleaned with surgical spirit.
Group 1 treated with poly herbal hair repair oil, Group 2 treated with hubun care oil, Group 3 mysha herbal hair oil, Group 4 served as negative control and Group 5 was given minoxidil 2% solution.
Approx.1 ml of each oil was applied per animal daily, and the treatment was continued for thirty days. Hair growth initiation time was observed and recorded i.e. the minimum time required to initiation of hair growth on shaved skin of the rats, hair growth completion time was recorded i.e. minimum time required for the complete hair growth to cover the shaved or denuded skin completely [4, 26].

Measurement of hair length
Hair was plucked randomly using sterile forceps from the depilated area on the 15th and 30th day of treatment and the hair length was measured with the help of a scale or Vernier caliper and the results were calculated as mean length ± SEM of 15 hairs [1, 27].

Stability studies
Each herbal hair oil was assessed under a controlled room temperature of 25 ± 2^{o}C and relative humidity of 60 ± 5%, to determine its stability over time, focusing on key parameters that influence quality and efficacy, which were Physical appearance, pH, Acid value, Saponification value, Specific gravity, Viscosity [4].

Physical and chemical evaluation of the formulated herbal oil:
The formulated herbal oil was assessed both physically and chemically to identify the phyto-constituents in accordance with Ayurvedic Pharmacopeial standards. This evaluation encompassed general characterization parameters such as pH, acid value, refractive index, specific gravity, color, and odor, as outlined in Tables 2, 3, and 4[26, 28]

Antioxidant assay (DPPH):

$$\frac{\text{Control abs. - sample abs.}}{\text{Control abs}}$$

According to the method of Naveed et al., a solution of 0.1mM DPPH in chloroform was prepared, which showed a characteristic purple/violet color. Sample solution, prepared in µg/ml concentrations, was added to 3ml of the DPPH solution at different concentrations (50, 100, 150 µg/ml levels) together with a standard ascorbic acid sample. Each experimental set was carefully performed in triplicate, with the addition of a blank sample to account for any effects of the colored material. After a complete incubation period of 30 minutes in the absence of light, absorbance readings were taken at 517nm using a spectrophotometer, giving precise results of antioxidant activity [29] [30]. DPPH % was calculated using the following formula:

DPPH % = x 100

RESULTS

Skin irritancy
In the biological evaluation of poly herbal hair repair oil, the product was subjected to dermatological testing (skin irritancy test) on animal skin to assess its safety and potential irritative effects. The results were promising: the oil did not induce any erythema (redness) or edema (swelling) in the skin of the test animals. This finding is significant as it indicates that the formulation is non-irritating and safe for use, suggesting that it is well-tolerated by the skin. Such outcomes are crucial for cosmetic products, particularly those intended for hair care, as they are often applied to the scalp, which is sensitive and prone to irritation.

Hair growth study
According to this study, all the oils performed well but Poly herbal hair repair oil was excellent in performance. It took less time for hair growth initiation and completion in animals treated with Poly herbal hair repair oil as compared to other oil samples (figure 1). The result of Poly herbal oil was comparable to the standard, as shown in Table 1.

Hair length measurement study
In terms of the hair elongation research study, the animals that were treated with Poly herbal hair repair oil showed pronounced hair length as compared to other oils. The observations of the study are recorded in Table 2, which again favors the use of Poly herbal hair repair oil as shown in Figure 1.

General characteristics
The results of the general characteristics or physicochemical evaluation, as shown in Table 3, are indicative of the fact that all the oils possess distinctive odors. The color of Hubun care and Mysha hair oils were found to be greenish brown while Poly herbal hair oil was of greenish yellow color. All the oils retained their intended appearance and odor throughout the study.

Physical parameters
The observed values for pH, specific gravity, acidic value, saponification value, refractive index and viscosity are given in Table 4. The values show that all the hair oils appear to align well with standard expectations for similar products. The absence of irritation and sensitivity suggests that the oils are suitable for sensitive skin, which is normally expected from herbal formulations.

Phytochemical screening
The analysis of different hair oil samples for the presence of prominent phytochemicals like alkaloids, flavonoids, glycosides, carbohydrates, saponins, proteins, vitamin C etc., is shown in Table 5. The various herbal constituents along with amazing phytochemicals present in poly herbal hair repair oil, may be the cause for the significant hair growth activity besides good antioxidant activity. All these agents not only show promising activity due to their natural origin but are also devoid of potential side effects as compared to synthetic drugs.

Anti-Oxidant study
Table 6, presents the antioxidant activity of various herbal hair oils, measured through a key parameter: DPPH scavenging activity. This parameter provides insights into the oils' ability to combat oxidative stress, which is crucial for maintaining healthy hair and scalp. DPPH Scavenging activity of poly herbal hair repair oil was found to be 73.50%, 60.33% for Hubun care herbal oil and 55.11% for mysha hair oil at a concentration of 150(µg/ml) respectively, of each oil. Poly herbal hair repair oil shows the highest DPPH scavenging activity, indicating strong antioxidant properties. It further

emphasizes its effectiveness in combating oxidative stress, making it an excellent choice for promoting hair and scalp health.

Stability studies

All oils retained their clarity and physical appearance throughout the study. The pH values of the oil samples showed slight fluctuations. Poly herbal hair repair oil maintained a stable pH of around 6.0, which is ideal for the health of hair and scalp, and shows that the formulation is well-balanced and unlikely to irritate. Poly herbal hair repair oil showed the least variation in acid value over time, suggesting barely any sort of degradation. However, this change was within acceptable ranges. In contrast, other oils showed more significant changes, which would indicate a higher tendency toward deterioration. All formulations showed consistent Saponification values, suggesting that the fatty acid content was adequately retained. Minimal variations in the specific gravity readings indicated that the oils' density was maintained. Consistent values for poly herbal hair oil show formulation stability, which is necessary to guarantee even application. All oils showed a minor loss in viscosity with time, however, the texture of Poly herbal hair oil remained appealing. A slight decrease in viscosity was noted in all oils over time, but Poly herbal hair oil retained a desirable texture. This suggests that this formulation is more robust than the others.

Discussion:

The primary skin irritation test showed that no edema or erythema occurred on the skin of the tested animals, showcasing that the formulations were completely non-irritanting. This suggests that all of the oil samples were safe to use and may be a positive indicator of their suitability for wider dermatological use.

In herbal hair growth studies, 30 shaved Sprague dawley rats were taken in 5 groups, 6 rats per group. The effect of hair growth was observed for one month . It was found that the Poly herbal hair repair oil increased hair growth compared to others after using the oil for 1 month and with no side effects. The poly herbal hair repair oil showed good hair growth promotion activity as the animals that were treated with this oil developed longer, denser, anagenic hair and took less time for hair to cover the denuded skin of experimental animals, compared to control and other test groups. Poly Herbal shows a hair growth initiation time of 6 ± 0.38 days, which is significantly faster than the control group (13 days) and comparable to the standard Minoxidil treatment (5 days). This suggests that it can effectively stimulate hair follicles early in the treatment process. The completion time for Poly Herbal is 21 ± 0.37 days, which is also quicker than the control group but slower than minoxidil and mysha herbal oil. However, it outperforms hubun care, which completes hair growth in 24 days. Overall, poly herbal hair repair oil stands out in this study for its balance of rapid initiation and effective completion of hair growth. Its significant results in both categories highlight its potential as a strong contender in the herbal hair care market, making it an excellent choice for individuals seeking an effective and gentle hair growth solution.

Poly herbal hair repair oil demonstrates substantial advantages in promoting hair length over a period of 30 days [31]. Its significant improvements in hair length at both the 15th and 30th days highlight its effectiveness as a hair growth treatment. According to the 15th-day assessment, Poly herbal hair repair oil-treated animals showed hair length of 21.50 ± 0.43 mm* (significant). Compared to the control group (13.11 mm), Poly herbal oil shows a marked improvement in hair length. It also performs better than mysha (17.06 mm) and is closer to hubun care (20.41 mm), exhibiting a greater early efficacy. According to 30th day hair length assessment, poly herbal oil treated animals showed hair length of 28.23 ± 0.41 mm* (significant). Again, poly herbal outperforms the control and shows significant growth, surpassing both hubun care (26.23 mm) and mysha (24.19 mm). It is just slightly behind minoxidil (30.17 mm), which is expected given minoxidil's established efficacy [32] [33]. By achieving results comparable to minoxidil and outperforming herbal counterparts, poly herbal emerges as a promising option for individuals seeking a natural and effective solution for hair growth.

In respect of physicochemical properties, the prepared poly herbal hair repair oil formulation was greenish yellow in color with pH (6.5) almost in accordance with human skin pH (6.8) which was neutral to slightly acidic. Hubun care herbal hair oil and mysha herbal hair oil were described as greenish brown**.** The odor of all herbal hair oils was characteristic. The difference in color compared to the other two oils may indicate a difference in the types or concentrations of herbal ingredients that have been added. A lighter yellowish tint could indicate that there is more of a certain oil or extract responsible for that color, which could influence consumer preferences.

Key quality and stability indicators for herbal hair oils include pH, acidic value, specific gravity, refractive index, viscosity, saponification value and skin irritation. The acidic value fell within acceptable limit (typically 5-15mg KOH/g for oils) for all oils, indicating a low level of free fatty acid [34] while the Poly herbal hair repair oil has the highest acidic value (13.80 mg KOH/g), this may indicate a stronger formulation that could be useful for certain types of hair, particularly those requiring extra care, nourishment and repair. Specific gravity measurement usually lies between 0.8-1 g/cm^3 for hair oils, signifying the density of formulations [35]. Poly herbal hair oil shows the lowest specific gravity value i.e, 0.83, indicating a lighter texture which could make it easier to apply and less prone to make the hair feel heavy. With a refractive index of 1.6634, Poly herbal hair repair oil may have unique light reflecting properties, potentially making hair appear shinier and healthier. The viscosity of hair oils usually ranges from 10-100 cps. The viscosity of Poly herbal hair oil formulation turns out to be 30cps (e.g., a balanced viscosity), which shows that it is neither too thick nor too runny; suitable for easy application. The saponification value of Poly herbal hair repair oil (26. 482mg KOH/g) is indicative of its cleansing potential while still providing nourishment.

Poly herbal hair repair oil stands out for its rich composition of beneficial phytochemicals. The combination of glycosides, saponins, flavonoids, proteins, vitamin C, and tannins makes it a comprehensive treatment for hair health. Saponins have natural cleansing properties and can help in removing impurities and excess oil from the scalp [36]. Known for their therapeutic properties, glycosides can promote scalp health and stimulate hair growth. They often have moisturizing effects [37]. Flavonoids are powerful antioxidants that protect hair and scalp from oxidative stress and environmental damage. They can also enhance blood circulation [38] [39]. Essential for strengthening hair strands, proteins help repair damage and improve overall hair texture [40]. Vitamin C is vital for collagen production, which strengthens hair and promotes healthy growth. It also helps in maintaining scalp health [41]. Tannins have astringent properties that can help tighten hair follicles and improve hair texture, providing a conditioning effect [42] [43]. Overall, poly herbal hair repair oil exemplifies the ideal qualities of a quality herbal hair oil, making it an excellent choice for individuals looking to enhance their hair's health and vitality.

The antioxidant activity highlights the strong protective qualities of poly herbal hair repair oil. Its comparable performance to the standard suggests that it is not only effective in promoting hair health but also in providing vital protection against oxidative stress. This positions poly herbal as a viable and attractive option for consumers seeking a natural hair care solution with significant antioxidant benefits. The DPPH scavenging activity indicates that the oil has substantial antioxidant properties, capable of neutralizing free radicals [44] [45] While it is significantly lower than ascorbic acid (90.0%), it is the highest among the herbal oils tested. When compared to the standard, our result suggests that the oil possesses notable antioxidant properties. The substantial antioxidant activity of poly herbal hair repair oil suggests that it may help in protecting hair from damage caused by environmental stressors, potentially improving hair health and vitality. The antioxidant property makes it an attractive ingredient for cosmetic formulations aimed at repairing and nourishing hair.

The stability study conducted on poly herbal hair repair oil over 30 days revealed that the formulation remained stable over a storage period of one month. It confirmed the resilience of herbal hair oil under ambient conditions, with minimal fluctuations in pH, viscosity, specific gravity and both acidic and saponification values remaining within acceptable ranges. Stability is a critical factor in product formulation, as it ensures that the product maintains its efficacy, safety, and overall quality over time [46]. The stability of the oil suggests several important points: The active ingredients within the poly herbal hair repair oil retained their antioxidant and therapeutic properties throughout one month, ensuring that consumers receive the intended benefits during this time. A stable formulation implies a longer shelf life, which is advantageous for both manufacturers and consumers. It reduces the risk of product degradation and enhances consumer confidence in the product's effectiveness over its lifespan [47]. The stability indicates that the formulation can withstand standard storage conditions without compromising its quality, making it practical for retail and consumer use.

Conclusion

The promising results of poly herbal hair repair oil highlight its potential as a natural, effective solution for promoting hair growth and improving scalp health. With its balanced pH, stability, and lack of side effects, this herbal formulation offers a safe and nourishing alternative to conventional hair care products. The findings of the study support the continued use of poly herbal hair repair oil, suggestive of maintaing its effectiveness, strength and quality over time. This not only revolutionizes the hair care industry but also provides a holistic approach to hair rejuvenation that harnesses the power of nature.

Authors contribution

Performed the experimental work: F. Gul, Helped in oil preparation and manuscript writing: F. Kanwal, Helped in animal activity: N. Ain and Y. Rehman, Helped in statistical analysis and data interpretation S. Khan and Z. Rahman, Helped in manuscript drafting and animal activity: S. din Badar, Supervised the project: I. Shah and M. Rehman, Helped in manuscript drafting and animal activity: A. Khan, G. Sajjad

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Conflict of Interest

No conflict of interest exists.

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Figures' legend

Figure 1: Hair growth study of the poly herbal growth promoting oil *vs* control

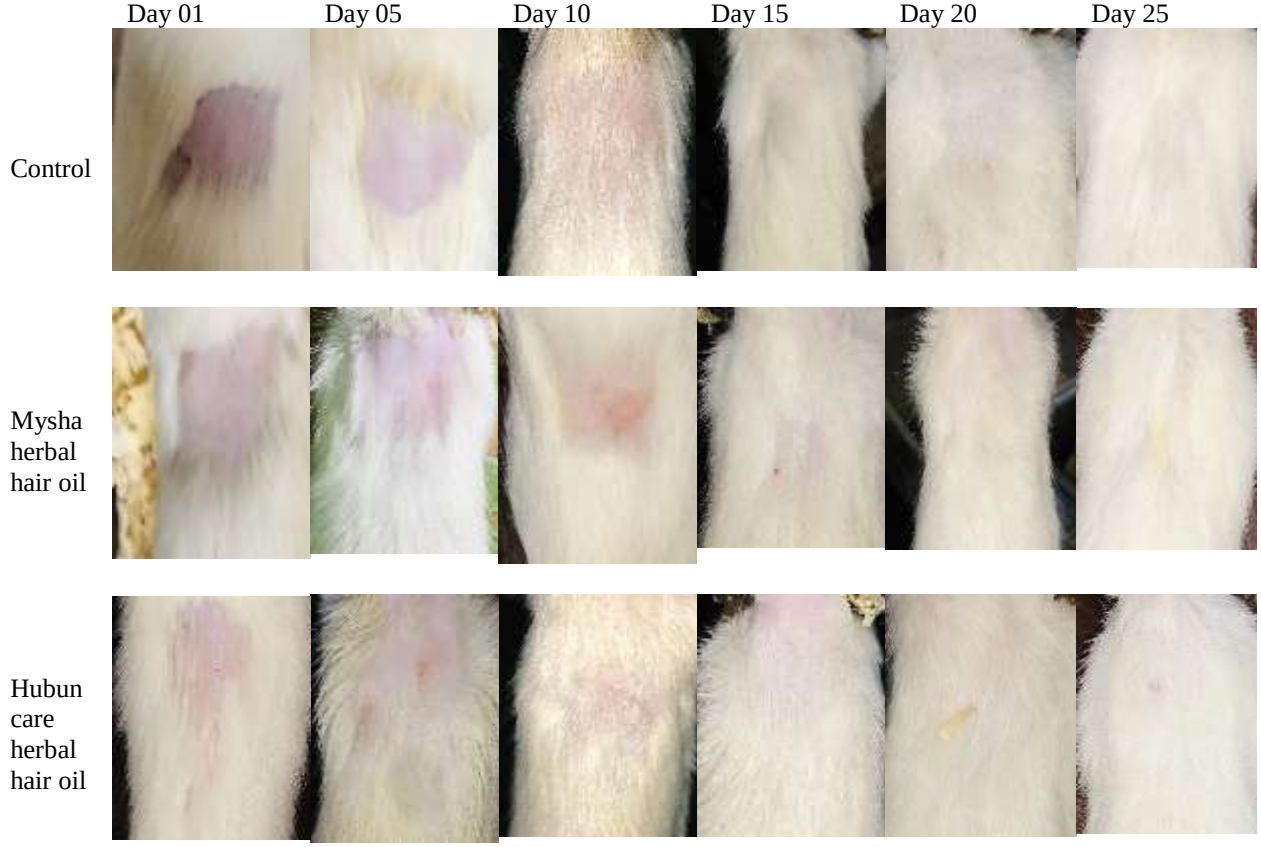

Figure 1: Hair growth study of the poly herbal growth promoting oil vs control

Table 1: Ingredients of the oil

S.#	Herbs for infusion	Additional herbs	Other oils
1	Tea tree leaves: 2-3%	Curry pata (*Murraya koenigii*): 3-4%	Coconut oil: 30-35% (base oil)
2	Rosemary leaves: 2-3%	Hibiscus flowers (*Hibiscus rosasinensis*): 3-4%	Olive oil: 20-25%
3	Monarda (Bee balm) leaves: 1-2%	Methidana (*Trigonella foenum*): 4-5%	Almond oil: 10-15%
4	--	--	Sesame oil: 5-10%
5	--	--	Castor oil: 2-5%
6	--	--	Rosemary essential: 2-3%
7	--	--	Peppermint essential oil: 0.5-1%

Table 2: Time taken for initiation and completion of hair growth

S.#	Groups	Treatment	Hair growth initiation (Day)	Hair growth completion (Day)
1.	I	Control	13 ± 0.67	28 ± 0.51
2.	II	Minoxidil (Standard)	5 ± 0.43***	17 ± 0.33***
3.	III	Hubun care herbal hair oil (Test Sample)	8 ± 0.37*	24 ± 0.45**
4.	IV	Mysha herbal hair oil (Test Sample)	10 ± 0.59**	26 ± 0.48*
5.	V	Poly herbal hair repair oil (Test Sample)	6 ± 0.38***	21 ± 0.37***

Data are reported as mean ± SEM. n= 06. *P<0.05, **P<0.1, and ***P<0.001.

Table 3: Observations for hair length measurement

S. No.	Group	Treatment/Sample	Hair length (mm)	
			15th Day	**30th Day**
1.	I	Control	13.11 ± 0.58	22.20 ± 0.67
2.	II	Minoxidil (Standard)	23.31 ± 0.41***	30.17 ± 0.33***
3.	III	Hubun care herbal hair oil (Test Sample)	20.41 ± 0. 65**	26.23 ± 0.58 *
4.	IV	Mysha herbal hair oil (Test Sample)	17.06 ± 0.59*	24.19 ± 0.53**
5.	V	Poly herbal hair repair oil (Test Sample)	21.50 ± 0.43***	28.23 ± 0.41***

Data are reported as mean ± SEM. N=15. *P<0.05, **P<0.01, and ***P<0.001.

Table 4: Evaluation of general characteristics of oil formulations

S. No.	Parameter	Hubun care herbal hair oil	Mysha herbal hair oil	Poly herbal hair repair oil
1.	Colour	Greenish brown	Greenish brown	Greenish yellow
2.	Odor	Characteristic	Characteristic	Characteristic

Table 5: Physical Parameters of Oil Formulations

Physical parameters	Oil Samples		
	Hubun care herbal hair oil	Mysha herbal hair oil	Poly herbal hair repair oil
pH	6.1	5.8	6.5
Acidic value (mg KOH/g)	11.39	10.42	13.80
Specific gravity(g/cm³)	0.91	0.95	0.83
Refractive index	1.5573	1.4650	1.6634
Viscosity (cps)	29	29	30
Saponification value (mg KOH/g)	23.482	19.536	26.482
Skin irritation	No irritation	No irritation	No irritation
Sensitivity test	No irritation	No irritation	No irritation

Table 6: Phytochemical screening of oil formulations

Name of Analysis	Hubun care herbal hair oil	Mysha herbal hair oil	Poly herbal hair repair oil
Alkaloids	-ve	+ve	-ve
Glycosides	+ve	+ve	+ve
Carbohydrates	-ve	+ve	-ve
Saponins	+ve	+ve	+ve
Flavonoids	+ve	-ve	+ve
Proteins	-ve	-ve	+ve
Vitamin C	-ve	-ve	+ve
Tannin	+ve	+ve	+ve
Terpenoids	+ve	-ve	-ve

Table 7: Antioxidant activity

S. No.	Sample	Sample concentration (µg/ml)	DPPH scavenging activity (%)
1.	Ascorbic acid (Standard)		90.0 ± 0.5***
2.	Poly herbal hair repair oil	50	54.40 ± 0.1
		100	61.13 ± 0.3*
		150	73.50 ± 0.4 **
3.	Hubun care herbal hair oil	50	43.21± 0.3
		100	49.02 ± 0.5
		150	60.33 ± 0.6*
4.	Mysha herbal hair oil	50	31.45 ± 0.1
		100	39.40 ± 0.7
		150	51.11 ± 0.5

Data are reported as mean ± SEM. N=15. *P<0.05, **P<0.01, and ***P<0.001.