RESEARCH ARTICLE

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Effectivity of Carrot (*Daucus carota*) Seed Oil as Hair Growth Promoter on Rats (*Rattus novergicus*)

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Abstract

Hair quality and health are important not only for the esthetical reasons but also to maintained the hair's function in protecting the head. Carrot seed oil (CSO) is rich in vitamin A and beta-carotene, which influence hair quality. CSO also contains carotol, which has anti-fungal properties that treat dandruff. This research aimed to observe the potential of CSO as a hair growth promoter. Twenty male rats were used in the study, with their backs shaved and divided into two areas, each with different frequencies of CSO application. The backs of the rats were given CSO every day for 14 consecutive days with different concentrations, i.e., 25%, 50%, and 75%. Hair growth was assessed based on its length, root, shaft, and tip thickness on days 9,12, and 14 and the results were recorded. On day 15, the rats were euthanized and their skin was collected to observe the number of follicles. Results showed that the application of CSO with different concentrations had no effect on hair length growth but it did affect the thickness of the root, shaft, and tip of the hair, with the best concentration being 75%. The frequency of application did not seem to affect the growth in terms of the length or thickness of the root, shaft, and tip of the hair; however, the application of CSO with 75% concentration and an application frequency of once a day produced the best amount of follicle regeneration.

Keywords: Carrot seed oil, hair growth, rat

Introduction

Thick, long, shiny, healthy, and manageable hair is a defining feature that enhances its owner's appearance. Hair plays a crucial role in personal care, as it boosts confidence in both men and women and provides protection for the scalp, shielding it from direct sunlight and potential impacts from external objects. Numerous products are available to improve hair growth and quality, one of which is hair growth promoters. Hair growth promoters are products designed to address issues such as hair loss while enhancing the beauty and quality of hair. These products are practical, easily absorbed by the scalp, and are formulated to minimize the risk of irritation.[1]

Synthetic hair products have been proven to have several undesirable side effects. For example, minoxidil is one of the infamous hair products and the most widely used as a hair growth promoter. Minoxidil has several unwanted effects, such as skin allergies, headaches, vertigo, edema, and hypotension. Due to the undesirable effects of synthetic materials, the concept of natural living and herbal usage has once again become the people's choice, which is also supported by Indonesia's wealth in natural resources.[2] Carrot seed oil (CSO) is rich in vitamin A, which is very important for maintaining the quality and healthiness of the hair. Besides vitamin A, CSO is also rich in beta-carotene (Pro-vitamin A), which protects the body from cell damage and nourishes the scalp and hair to keep them healthy.[3,4] Apart from being able to strengthen and maintain hair quality, CSO also contains carotol, which has antifungal properties that can be used to treat dandruff. Carrot is rich in flavonoids. Flavonoids have been proven to increase the regeneration of hair follicles, which can help to accelerate hair

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growth.[5] There are several studies regarding hair growth promoters using other herbs such as Siberian geranium (*geranium sibiricum L.)*[6], olive *(olea europaea L.),*[7] Gingseng *(Panax ginseng Mayer)*[8], Alder *(Alnus sibirica Fisch. ex Turcz)*[9], *Lotus seeds (Nelumbinis semen)*[10], and brown alga (*Ishige Sinicola*)[11]. However, currently there are no research conducted on carrot seed oil in affecting hair growth, specifically with different frequencies.

Therefore, this research aims to investigate the potential of carrot seed oil (CSO) as a hair growth promoter. In addition to evaluating hair growth, the study also assessed hair quality using parameters such as the thickness of the hair root, shaft, and tip, as well as the proliferation of hair follicles. It is anticipated that this research will provide scientific evidence regarding the efficacy of CSO as a hair growth promoter and highlight its potential as an alternative natural ingredient for promoting hair growth.

Methods

This research was conducted at the Laboratory of Pharmacology and Toxicology and the Laboratory of the Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University, from February to April 2023. The study adhered to the ethical guidelines set by the Committee of Animal Ethics, Faculty of Veterinary Medicine, Bogor Agricultural University, and received ethical clearance under certificate number 025/KEH/SKE/III/2023. The equipment used in this study included latex gloves, cotton buds, tweezers, and 250 mL plastic containers. The materials involved were twenty male rats (*Rattus norvegicus*) weighing 30–40 g, anthelmintic ivermectin, depilatory cream, and carrot seed oil, all commercially sourced from a marketplace.

The preparation stage begins with acclimatization at Laboratory of Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University. Acclimatization lasted seven days to ensure the rats could adapt to the environment and reduce stress. The room and the cages were cleaned first before the placement of the rats. Rats that will be acclimatized must be in appropriate conditions. Based on Nugroho,[12] the normal temperature for mice ranges from 18–26°C, with relative humidity of 40–70%,[13] and lighting settings of 12 hours of light and 12 hours of darkness.[14] Five rat cages made of plastic were used, measuring 55 Cm x37 xCm x17 cm, equipped with a 500 mL drinking bottle, wood shavings as a base, and cage covers. Mice were given food in 10% of their body weight. Cage bedding was replaced once every seven days. Rats were fed after 1-2 hours after treatment.[15]

The treatment was carried out after acclimatization. CSO was applied for 14 days by dipping a cotton bud and applying it to the treatment area. The treatment area was created by depilating the hair with depilatory cream. The treatment area was the rat's back (Figure 1). The back was divided into two parts, the dorsal area, and the caudal area. The dorsal area or Area 1 (A1) was an area with a frequency of application once a day, while the caudal area or Area 2 (A2) was an area with a frequency of application twice a day. Treatment was started a day after hair removal with depilatory cream to avoid the effects of skin irritation. Mice were divided into four groups (n=5), as follows:

The parameters assessed in this study included hair growth in terms of length, root, shaft, and tip thickness, as well as the number of hair follicles. Rat hair samples were collected on days 9, 12, and 14 of the study to evaluate the length and thickness of the root, shaft, and tip. Measurements were taken by gently pulling the hair from the root and attaching it to a glass slide. The hair was then examined under a microscope at 40x magnification. At the conclusion of the study, the rats' skin was harvested. Anesthesia was induced using a chemical method, with ketamine (100 mg/kg body weight) and xylazine (80 mg/kg body weight), administered via the intraperitoneal route. Once the rats were anesthetized, blood was collected through the intracardiac method. Subsequently, the dorsal skin was excised by snipping. The skin samples were fixed in 10% buffered neutral formalin (BNF) for histopathological analysis, and Haematoxylin and Eosin staining was applied. Hair follicles were examined in histopathological sections under a microscope at 40x magnification. All measurements and counts were performed using ImageJ software. Data were tested for normality and homogeneity, followed by Kruskal-Wallis analysis. Data analysis was conducted using R software.

Results

Figure 2 shows significant differences between TW1, TW2, TW3, and TW4 regarding hair root thickness. These significant differences in each

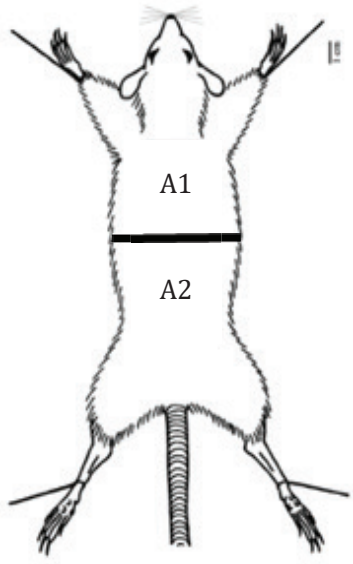

Figure 1 Schematic View of Dorsal Rats Was Given the CSO Treatment
A1. Dorsal area with once-a-day treatment; A2 is a caudal area with twice-a-day treatment

Table 1 Treatment Group of Testing the Effectiveness of Carrot Seed Oil (CSO) as *Hair Growth Promoter*

Group	Concentration	Treatment
TW1	Negative control	A1 (Dorsal area)
		A2 (Caudal area)
TW2	CSO 25%	A1 (Dorsal area)
		A2 (Caudal area)
TW3	CSO 50%	A1 (Dorsal area)
		A2 (Caudal area)
TW4	CSO 75%	A1 (Dorsal area)
		A2 (Caudal area)

group show the influence of CSO concentration on the thickness of the hair root, with the TW4 group having the best hair root thickness, followed by TW3, TW2, and TW1 on days 9,12, and 14 (A). Regarding hair tip thickness, a significant difference was found between the

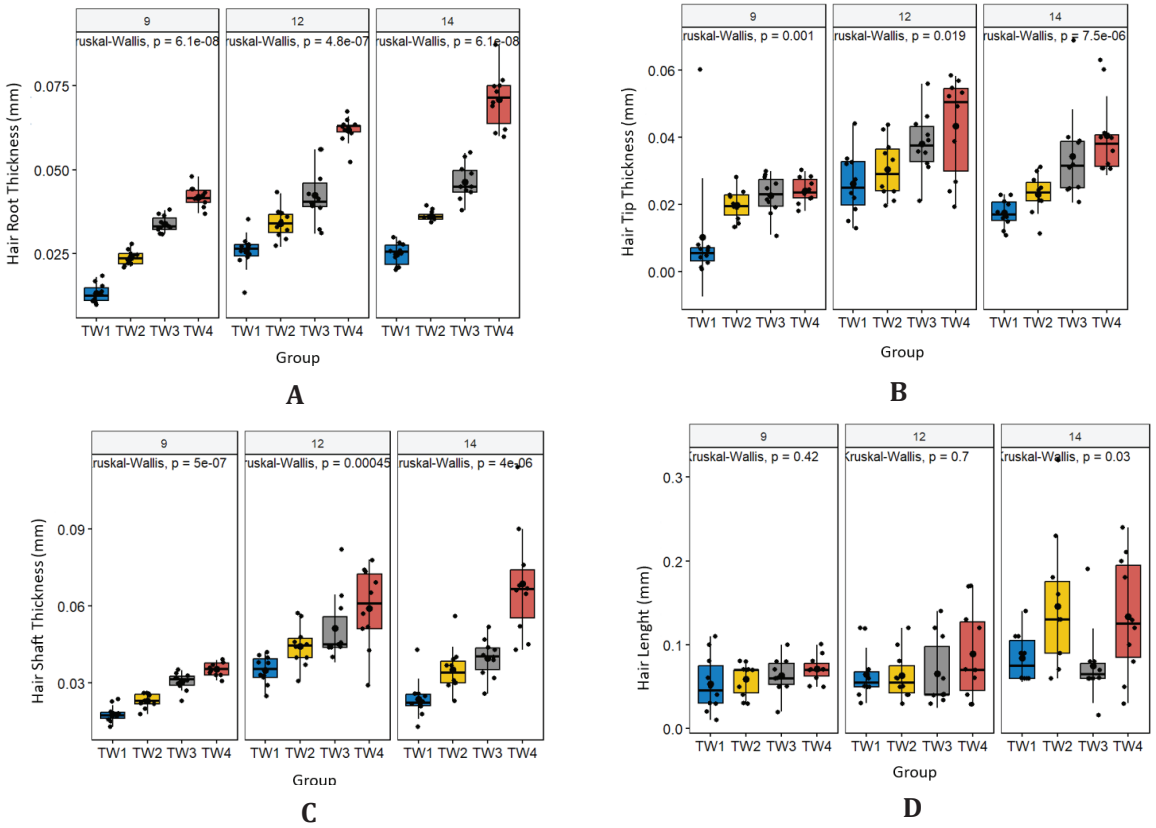

Figure 2 Effect of CSO Treatment to A. Hair Root Thickness, B. Hair Tip Thickness, C. Hair Shaft Thickness, and D. Hair Length in Serial Sampling at Days 9, 12, and 14. Significance ($p<0.05$)

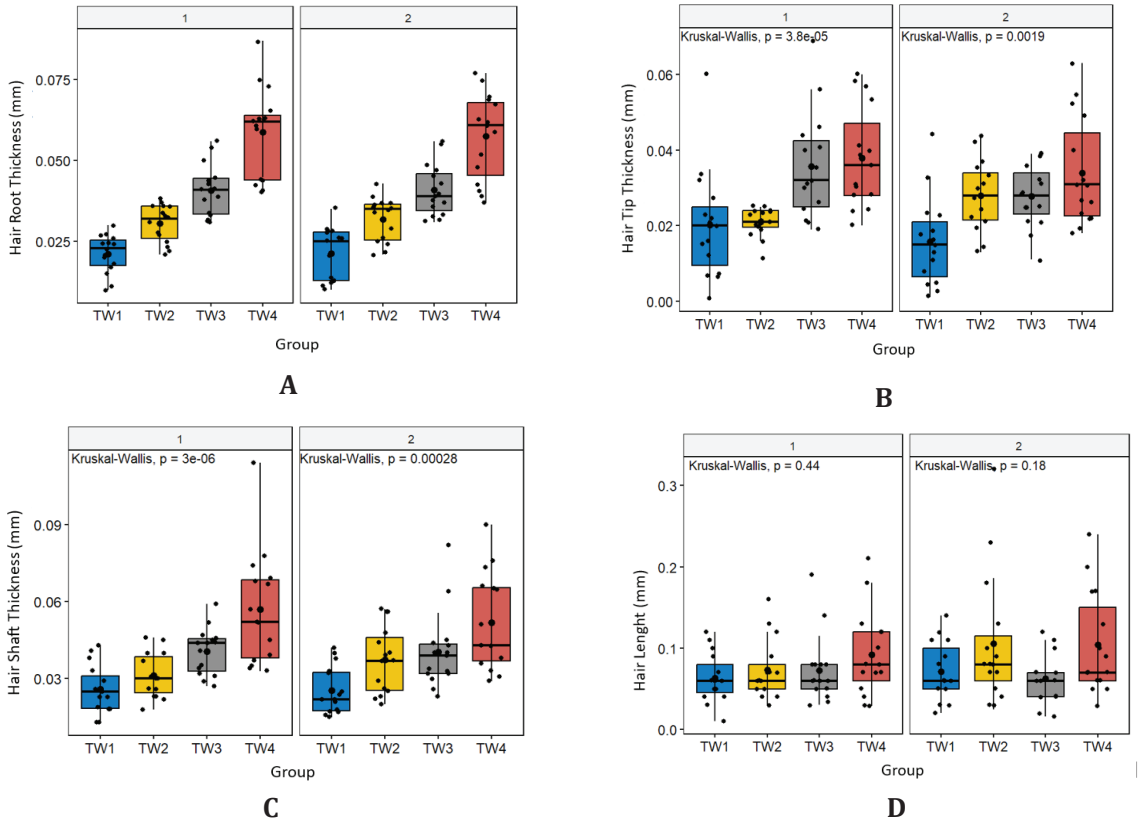

Figure 3 Effect Of CSO Treatment Frequency On (A) Hair Root Thickness, (B) Hair Tip Thickness, (C) Hair Shaft Thickness, and (D) Hair Length Based on Once- And Twice-Daily Treatments. Significant Differences Are Indicated by p<0.05

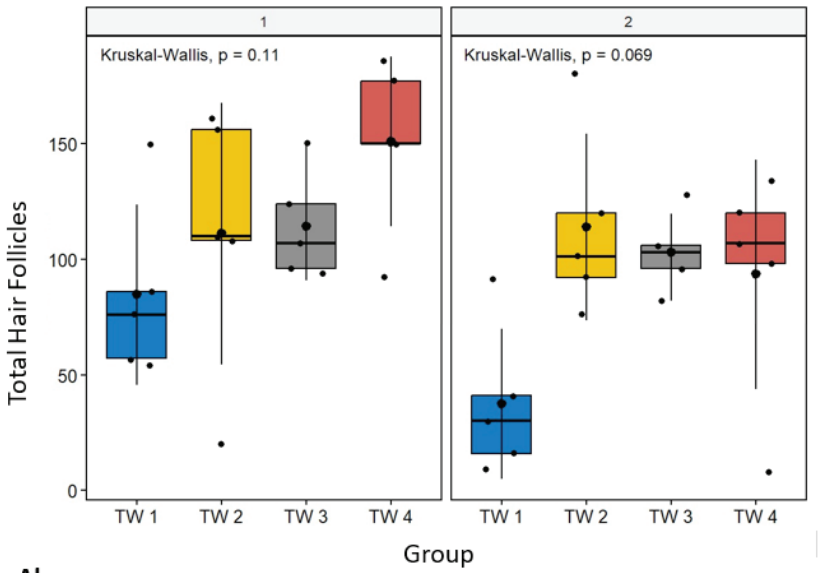

Figure 4 Effect of CSO Concentration and Application Frequency on the Number of New Follicles in Rat Skin with Once- and Twice-Daily Treatments. Significant differences are indicated by p<0.05

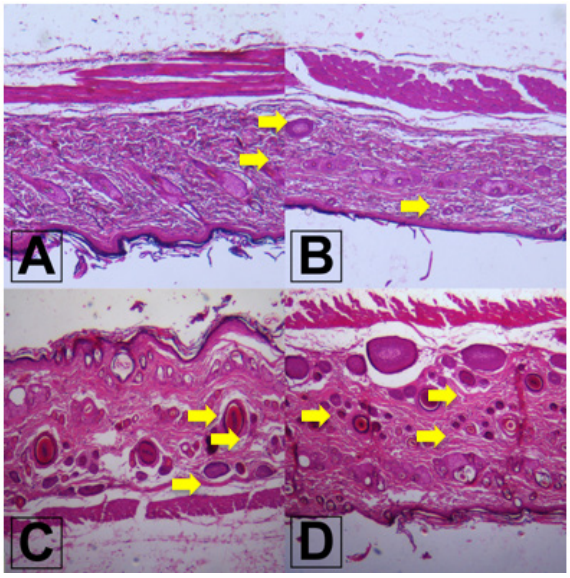

Figure 5 Histopathological Appearance of Rat's Skin with Hematoxylin and Eosin Staining. (A) TW1 Group, (B) TW2 Group, (C) TW3 Group, and (D) TW4 Group were Given Once-Daily Treatment with CSO. The Arrow Indicates Hair Follicles.

TW1 group and the TW3 and TW4 groups. A significant difference was also found in TW2 and TW4. TW4 shows the best hair tip thickness compared to the other groups (B). A significant difference was found between the TW1 group and the TW3 and TW4 groups regarding hair shaft thickness. Significant differences were also found between TW4, TW3, and TW2. This shows that concentration affects the thickness of the hair shaft, with the best hair shaft thickness shown by TW4 (C). However, no significant difference was found in the correlation of

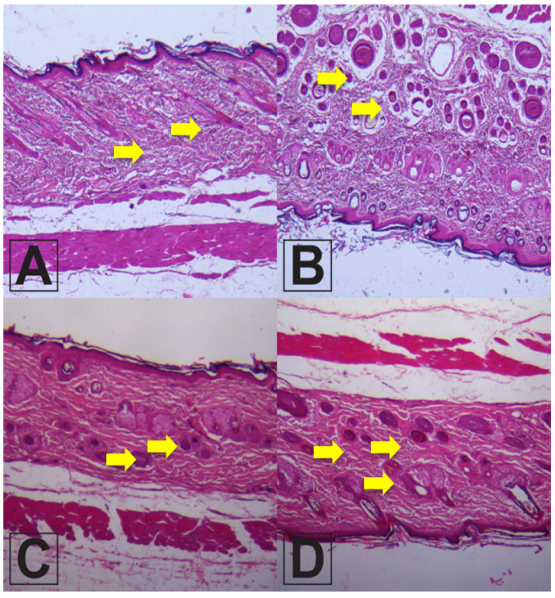

Figure 6 Histopathological Appearance of Rat's Skin with Hematoxylin and Eosin Staining. (A) TW1 Group, (B) TW2 Group, (C) TW3 Group, And (D) TW4 Group Were Given Twice-Daily Treatment With CSO. The Arrow Indicates Hair Follicles.

concentration and hair length parameters.

Based on Figure 3, no significant difference was found between A1 and A2 in hair root, hair tip, hair shaft thickness, and hair length. This suggests that the frequency of application does not affect hair quality.

Based on Figure 4, a difference was found in the number of follicles between treatments, with the highest number of follicles in TW4, followed by TW2, TW3, and TW1. A difference was also found in the effect of frequency of application, with single use showing the best effect.

Discussion

Hair loss and poor hair quality have become increasingly common issues in both men and women. According to the results of this study, carrot seed oil (CSO) was found to improve hair quality but did not stimulate hair length growth. Specifically, the application of CSO to rat skin increased the thickness of the hair root, shaft, and tip, as well as the number of hair follicles.

No significant differences were observed between the groups with varying concentrations of CSO regarding hair length growth in rats (Figure 2), nor was there a significant effect related to the frequency of application (Figure 3). These findings suggest that CSO does not influence hair growth in terms of length. However, significant differences were observed in the thickness of the root, shaft, and tip of the hair when different concentrations of CSO were applied, indicating that CSO improved the overall quality of rat hair (Figure 2). Furthermore, no significant differences were found in the thickness of the hair root, shaft, or tip when CSO was applied with different frequencies, suggesting that the frequency of CSO application does not affect hair quality (Figure 3).

Based on the follicle data obtained (Figure 4) differences were found between groups, with TW4 as the group with the largest amount of follicle numbers, followed by TW2, TW3, and TW1. The frequency of administration showed a significant difference, with the once-daily administration frequency group (A1) having a more significant number of follicles compared to the twice-daily administration frequency group (A2) (Figure 4)

The number of follicles on once daily application frequency being greater than twice daily shows that the effect of repeated application of CSO does not positively influence follicular regeneration. The difference in the frequency of CSO administration did not affect length growth or improve hair quality. However, the frequency of once-daily administration shows the regeneration of a greater number of follicles. This indicates that CSO has the ability to increase follicle regeneration with a certain capacity. Application of CSO beyond the maximum capacity will not show any greater effect on follicular regeneration. This is due to follicular saturation caused by CSO. Saturated hair follicles can slow down regeneration ability and cause a decrease in hair growth.[16]

Carrots (*Daucus carota* L.) are popular as vegetables and are also famous as cooking ingredients. Carrots are a plant in the family of umbelliferae Apiaceae. Carrots were originally used as a medicinal ingredient before becoming popular as a cooking ingredient. Carrots are rich in vitamin A which contains beta-carotene, functioning as a protector of the body from cell damage, can maintain healthy eyes and skin, and nourish the scalp and hair to keep it healthy.[2] Carrot preparations are widely used in cosmetics, and one of the carrot preparations that can be found commercially is carrot seed essential oil.

Essential oils have been widely used in hair cosmetics because of their bioactive content. Several essential oils, such as hair loss and dandruff, have been used to treat scalp problems. Carrot seed essential oil is widely used in various cosmetic applications due to its unique formulation. Carrot seed essential oil is widely used in various cosmetic applications due to its unique formulation. Carrot seed essential oil contains many fatty acids, with the highest composition being petroselinic acid (59.35%), followed by linoleic acid (11.82%), palmitic acid (10,01%), and stearic acid (2.41%). In contrast, other fatty acids such as palmitoleic, arachidonic oleic, and gadoleic acid are present in small amounts. The main components found in carrot seed essential oil are carotol (49.51%) and β-caryophyllene (47.99%).[17]

One of the highest ingredients in carrot seed oil, linoleic acid, has been proven to have hair-growing properties and can improve follicle regeneration and hair quality by increasing the expression of cell protein cycles such as cyclin D1 and cyclin-dependent kinase. Application of linoleic acid can also increase several growth factors, such as keratinocyte growth factor and vascular endothelial growth factor, in a dose-dependent manner. In addition, the fatty acid content of linoleic acid can help improve hair growth. Linoleic acid derivates maintain the stability of hair follicles by maintaining lipid

metabolism in hair and hair follicles.[18] Oleic acid, which is another fatty acid content in carrots, has been proven to prevent dermophytes and has an effect of supporting hair quality. Oleic acid can also maintain scalp health.[19] Also, based on several studies, one of the main ingredients in carrot seed essential oil, β-caryophyllene, has been shown to increase −hair follicle conversion. β-caryophyllene can also increase cell proliferation.[20]

Hair follicles are crucial components in hair growth. Matrix cells that continue to increase at the root of the hair-trigger hair growth. Differentiation of the follicles and the upward movement of the follicles cause hair shaft growth. Dermal papillae, located at the base of the follicle, control the number of matrix cells and the thickness of the hair. Hair growth is a continuous process. This cycle consists of four stages: anagen, catagen, telogen, and exogen. Each hair follicle goes through its cycle, with each hair follicle going through approximately 10-30 cycles in its lifetime.[21] The anagen cycle is the longest, lasting two to eight years in humans. The growth of the hair shaft from the follicle characterizes this phase. Change from the anagen phase to the telogen phase causes decreased hair growth. This phase is followed by a resting phase, where hair stops growing and continues with a phase of hair loss. Regeneration occurs from the telogen to the anagen phase. Hair follicle regeneration depends on activating follicular stem cells prominently under the sebaceous glands.[22] Carrots obtain flavonoids kaempferol, quercetin, and luteolin that can promote the growth of follicle of the hair.[23] Flavonoids are polar compounds that strengthen capillary walls and increase blood flow to hair follicles to stimulate the telogen to the anagen phase, thus triggering follicle regeneration and hair.[24] The application of certain preparations to the area of hair follicles can stimulate follicle regeneration. Increased follicle regeneration leads to enhanced hair growth, strength, and quality, including improvements in hair thickness and volume. However, this study had limitations, such as restricted resources, preventing identification of the specific component(s) of carrot seed oil (CSO) responsible for improving hair quality. Additionally, more resources were needed to measure anagen and telogen phase lengths and follicle density.

Based on the study, CSO treatment can enhance both hair growth and quality, as evidenced by significant differences in the thickness of the hair root, shaft, and tip in the groups treated with CSO compared to the negative control. The optimal concentration of CSO identified in this study was 75%. Although the frequency of CSO treatment did not significantly impact hair growth or quality, a once-daily application was found to promote follicle growth more effectively than twice-daily treatment. Further research is necessary to identify the specific components of CSO that contribute to hair quality and follicle proliferation, thereby maximizing its potential as a promoter of hair quality.

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