



ISSN Print: 2664-9926  
ISSN Online: 2664-9934  
Impact Factor: RJIF 5.45  
IJBS 2020; 2(2): 57-61  
[www.biologyjournal.net](http://www.biologyjournal.net)  
Received: 22-05-2020  
Accepted: 02-07-2020

**Kalim Ullah**  
Department of Zoology  
Government Post Graduate  
College Karak, Khyber  
Pakhtunkhwa, Pakistan

**Azra Azam**  
Department of Zoology  
Government Post Graduate  
College Karak, Khyber  
Pakhtunkhwa, Pakistan

**Akhter Khan**  
Department of Zoology  
Government Post Graduate  
College Karak, Khyber  
Pakhtunkhwa, Pakistan

**Shafi Ullah Gul**  
Lecturer at Department of  
Zoology Government Post  
Graduate College Karak,  
Khyber Pakhtunkhwa,  
Pakistan

**Ayesha Ihsan Qazi**  
Khyber Medical University  
Peshawar, Khyber  
Pakhtunkhwa, Pakistan

**Corresponding Author:**  
**Kalim Ullah**  
Department of Zoology  
Government Post Graduate  
College Karak, Khyber  
Pakhtunkhwa, Pakistan

## Effect of topical applications of oils extracted from medicinal plants and coconut oil mixed with egg yolk and vitamin e on growth of rabbit hair of District Karak, Khyber Pakhtunkhwa, Pakistan

**Kalim Ullah, Azra Azam, Akhter Khan, Shafi Ullah Gul and Ayesha Ihsan Qazi**

**DOI:** <https://doi.org/10.33545/26649926.2020.v2.i2a.57>

### Abstract

Most people are unaware of how to properly care for their own hair and scalp. Healthy hair and a healthy scalp usually go hand in hand, so a good health scalp is required to give the look of hair health and vice versa. Hair loss is caused by genetic and hormonal changes, but weathering and pampering habits also have an impact on our hair. The purpose of this study was to look into the healthy hair quality dimensions of oil extracted from Amla, rittha, and sikha Kai, as well as the effect of coconut oil made by mixing with egg yolk and vitamin E in male rabbits. Five healthy adult male rabbits were included in this study. Plant oil were extracted by direct boiling method while coconut oil was mixed with egg yolk (1:1 ratio) and vitamin E. Animals hair were trimmed at three points (back and lateral sides) with razor blades (1x1 inch each) on experimental day one. The points were randomly allocated for oil application. Points were made on same animal to avoid animal to animal variation and randomly, to avoid point to point variation of hair growth. Three groups were made for the experiment, first group served as control and no oil was applied to those points. In the second group, Amla, reetha and Sikhakai extracted oil was applied and was named T<sub>1</sub>. While in the third group, Coconut oil, Egg yolk and Vitamin E formulation was applied and was name T<sub>2</sub>. Eye irritation test and skin irritation tests were also done before the start of experiment. Oils showed no signs of irritation in eyes and on skin in rabbits. The majority of people are unaware of how to commit to making for their scalp and hair. Healthy hair and a good health scalp generally supplement each other, so a better and healthier scalp is required to give the aesthetic of hair health and vice versa. Weathering and grooming habits, as well as genetic and hormonal changes, play a role in hair loss. The current study was designed to look into the healthy hair future expansion of oil extracted from Amla, rittha, and sikha Kai, as well as the impact of coconut oil made by mixing with egg yolk and vitamin E in male rabbits. The results of the present study show that coconut oil supplied with egg yolk and vitamin E can enhance growth of the hair more efficiently however, its effect needs to be further analysed.

**Keywords:** Tropical, application, oils, medicinal, plants, coconut oil, egg yolk, Vitamin E, rabbit hairs and karak

### 1. Introduction

Although a healthy hair and hair are critical, the wider populace is unaware of these issues. Most people are unaware of how to properly care for one's hair and scalp. Healthy hair and a healthy scalp usually go hand in hand, so a good health scalp is required to give the aesthetic of hair growth and conversely. A good health scalp is commonly described by the absence of illness or abnormalities; however, a person can be bald and still be considered to have a healthy scalp. Dermatologists (Rogaine®, 2009; Monselise *et al.*, 2017) <sup>[1]</sup> concur that the features of hair health include thickness, volume, lustre, and fibre resilience. A healthy scalp, according to the majority, would have regular follicular density and no scaling, itching, burning, or erythema. Many clients who are experiencing hair loss believe the issue is related. They believe that treating the scalp will promote prospective hair growth, but this is not always the case.

Hair growth is cyclical, with growth (anagen), ingrowth (catagen), and rest (telogen) phases (Uno, 1986; Montagna, 2012; Montagna and Ellis, 2013) <sup>[2]</sup>. Complex notifications between the epithelial tissue and the dermis regulate the active development and rest cycles, which are still poorly understood. In a healthy scalp, the majority of follicles are starting to grow

(90 to 95 percent), a few are changes in major (less than 1 percent), and the remaining are resting (5 to 10 percent), (Headington, 1993; Whiting, 1996; Blume-Peytavi *et al.*, 2008) [3]. The hair is set to release and shed at the climax of telogen, and then the next cycle begins. Every day, up to 100 telogen hairs are dropped from the head, and roughly equal numbers of follicles gain entry anagen. The length of hair is determined by the duration of anagen, and the diameter is determined by the quantity of the hair bulb. We are birthed with all of our end hair follicles about 100,000 on the scalp – predisposed to keep growing, thick hair. Other follicles are predisposed to produce mucous neck hair, which really is short, fine, and nonpigmented, and covers a large portion of the body. Follicles can grow larger or smaller as a result of local and systemic influences that change the period of anagen and the loudness of the hair follicle matrix.

Androgenetic alopecia is a hereditary hair thinning caused by androgens in men and women who are genetically predisposed to it (Hamilton, 1942; Otberg *et al.*, 2007) [4]. In men, this is recognized as men hair loss or prevalent baldness, and in women, it is known as female-pattern hair loss. Hair thinning typically occurs between the ages of 12 & 40 years across both sexes, and roughly half of the population exhibits this trait to some degree before the age of 50. (Hamilton, 1951; Piraccini and Alessandrini, 2014) [5]. The inheritance pattern is broad range (Küster and Happle, 1984) [6]. Dihydrotestosterone works by binding to the androgen receptor in vulnerable follicle of the scalp, and the endocrine complex then stimulates the genes that code for the transformation of large hair follicles, terminal follicles to miniaturized follicles (Messenger and Sinclair, 2006) [7]; Yazdabadi *et al.*, 2008) [7, 8]. With each hair cycle, the length of anagen lessens and the follicles shrink, resulting in shorter, finer hair strands that encompass the scalp poorly. Androgenetic alopecia is distinguished by these miniaturized hairs of varying lengths and diameters (Hamilton, 1951; Otberg *et al.*, 2007) [4]. Hair suffers from aggression; some ailments to regular hair health can cause problems. The most common hair problems include pigmentation issues (fading) and hair loss (Shedding). Minoxidil, a synthetic drug that is a potent vasodilator, appears to be safe for long-term use.

After five years of using 2% and 3% pertinent minoxidil, the advancement has shown to climax at one year, with a steady decrease in regrowth over the subsequent years. Lengthy treatment with local side effects may be an issue if minoxidil lotion is used indefinitely (Wilson *et al.*, 1991) [12]; Bhalerao and Solanki, 2002) [12, 9]. Based on a market survey of crude drugs currently used for herbal hair oils, we can make a decision on which drugs to use for hair oil. As a result, the current study sought to assess the natural hair action of herbal formulations containing oil extracts of all of the drugs mentioned in varying concentrations.

A wide variety of herbs have been used in hair treatments. Amla, henna, neem, methi, lemon, tulsi, brahmi, shikakai, reetha, liquorice root, musk root, mahabhringraj, jantamasi, chitraka, marigold, hibiscus, nutmeg, parsley, rosemary, herb are a few of these herbs (Poucher, 2013) [10]. Minoxidil, a synthetic drug that is a potent vasodilator, appears to be safe for long-term use. After five years of using 2% and 3% controversial minoxidil, the progress was shown to peak at one year, with a gradual decline in regrowth over the following years (Olsen *et al.*, 2004) [11]. Lengthy care with

municipal side effects is an issue if minoxidil lotion is used indefinitely (Wilson *et al.*, 1991) [12]. Based on a market survey of crude drugs currently used for herbal hair oils, we can make a decision on which drugs to use for hair oil. As a result, the current study sought to assess the natural hair action of herbal formulations containing oil extracts of all of the drugs mentioned in varying concentrations. To back up the traditional claims, multi-ingredient hair oils are now prepared and tested for hair growth activity.

The plant Amla is a member of the Euphorbiaceae family. Amla is high in vitamin C and contains a significant amount of pectin, which is high in minerals such as phosphorus, iron, and calcium (Wilson *et al.*, 1991) [12]. Bacopa monnieri, a member of the Scrophulariaceae family, is known for its action on the brain, earning it the moniker "nervine tonic." Bacosides A and B have been shown to increase protein kinase operation and specific protein synthesising in cells. As a result, it is also used to treat dementia (Wagner and Bladt, 1996) [13]. Trigonella foenum-graecum is a member of the Leguminosae family and is used as a high protein fodder as well as for cleansing and softening properties. It improves scalp health and inhibits hair loss (Adhirajan *et al.*, 2003) [14]. Murraya koenigii leaves, which belong to the Rutaceae family, are used for their antiseptic properties (Kumar and Jayaveera, 2014) [6]. Cocus nucifera, a member of the Palmae family, is said to advertise hair growth.

Amla is high in vitamin C, tannins, and minerals like phosphorus, iron, and calcium, which nourish the hair while also darkening it (Wagner and Bladt, 1996) [13]. Hibiscus contains calcium, phosphorus, iron, vitamin B1, riboflavin, niacin, and vitamin C, and is used to promote thicker hair growth and prevent premature greying (Monselise *et al.*, 2017) [1]. Brahmi contains alkaloids that increase the activity of protein kinase (Otberg *et al.*, 2007) [4]. Methi contains a high protein fodder, which provides hair with the necessary protein nutrition (Evans, 2009) [16].

## 2. Materials and Methods

### 2.1 Study Specie

Five healthy male rabbits weighing 700-900 g were included in this study. Animals were purchased locally and were kept in separate steel cages in the department of Botany, Government Post Graduate College Karak, KPK, Pakistan. Animals were fed with rabbit diet and fodder and water was available *ad libitum*. These rabbits were acclimatized to handling for one week before the start of the experiment.

### 2.2 Preparation of herbal oil

The oil was prepared at the Department of Botany, Government Post Graduate College Karak, KPK, Pakistan. The formulation is as under.

### 2.3 Plant Material

Seeds of grape (*Phyllanthus emblica*), fruit of *Sapindus mukorossi*, also recognised as Reetha, and materials of *Senegalia rugata*, also known as Shikakai in India, were obtained from the local market and identified by comparing them to standard herbarium specimens at the Department of Botany GPGC Karak. The various plant drugs are squashed in a mixer and then passed through a sieve number 80.

### 2.4 Preparation of oil

To obtain the oil, the crude meds were powdered, weighed, and directly cooked in olive oil with vigorous agitation and

heaters until the drug was completely retrieved in the oil base. The olive oil was purchased at a local market and mixed with an egg yolk in a 1:1 ratio before adding vitamin E 40 mg/100 ml to the mixture. The oils were positioned in clean glass bottles for the experiment.

### 2.5 Eye irritation test

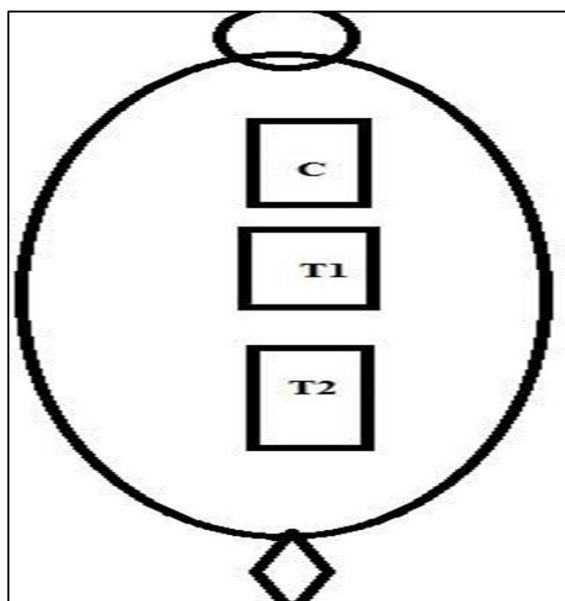
For eye irritation test, a drop of oil was put in the eye of each rabbit and eyes were observed for next 24 hours. Redness and swelling in the eyes were observed to know whether the oil causes any irritations. Each oil was individually checked for this test.

### 2.6 Skin irritation test

The oils were individually tested for skin irritation in the animals. Oil were individually applied to rabbit skin and were observed for next 24 hours for any kind of redness or any other sign of irritation.

### 2.7 Experimental design

Animal's hair were trimmed at three points (back and lateral sides) with razor blades (1x1 inch each) on experimental day one. The points were randomly allocated for oil application. Points were made on same animal to avoid animal to animal variation and randomly, to avoid point to point variation of hair growth. Three groups were made for the experiment, first group served as control and no oil was applied to those points. In the second group, Amla, reetha and Sikhakai extracted oil was applied and was named T1. While in the third group, Coconut oil, Egg yolk and Vitamin E formulation was applied and was name T2.



**Fig 1:** Spots selected for different treatments along the vertebral column of rabbit

The points were marked on each animal and oil was applied daily to the spot for next 48 days. Hair samples were collected weekly. Hair collected were used for length measurement and for microscopic studies of health and diameter. Hair were straightened and length was measured using roller and the length in millimeter or centimeter was reported. Photomicrographs were obtained and diameter was measured by ocular micrometer at 4x, 10x or 40x.

After the collection, hair were straightened and length was measured using roller and the length in millimeter or centimeter was reported.

### 2.8 Statistical analysis

The results were presented as Mean SEM. The results were statistically compared using one-way variance analysis (anova followed by Tukey's test. A P value of < 0.05 was deemed significant. P value less than 0.05 was considered significant.

### 3. Results and Discussion

Hair growth has been shown to be dependent on the mitotic index of hair bulbs in follicular (Montagna and Ellis, 2013)<sup>[2]</sup>. Thus, substances that increase the uptake of glucose and oxygen, thereby raising the supply of energy to the hair bulbs, have been noted to stimulate hair growth. Similarly, it has been reported that local application of vitamin C, which improves circulation from around hair bulbs, promotes hair development in rabbits (Kamimura and Sasaki, 1965). Sunlight, environmental temperature, feed, and age were also found to influence rabbit hair growth. Furthermore, head hair is infrequent and varies between individuals to some extent. However, whenever the rabbits are kept under similar management and environmental conditions and the sites of application are made on both sides of the vertebral column with variable sites of implement, these irregularities can be overcome (Ryder, 1958).

In the present study different treatment were checked for eye irritation and skin irritation for 72 hours. After application, the eyes and the skin were carefully checked and it was found that the treatments used in the presents study caused irritation neither in the skin nor in the eyes of the animals (Table 1).

**Table 1:** Results of eye irritation and skin irritation test of different treatments in rabbit

Para meters	R1	R2	R3
Eye Sensation	Not	Not	Not
Skin irritation	Not	Not	Not

Daily topic provides of plant derived oil promoted rabbit hair growth (P0.05) compared with untreated power at the end of the first week, which continued until the end of therapy in the seventh week. Hibiscus contains calcium, phosphorus, iron, vitamin B1, riboflavin, niacin, and vitamin C, and is used to promote thicker hair growth and prevent premature greying (Adhirajan *et al.*, 2003)<sup>[14]</sup>. Brahmi contains alkaloids that increase the activity of protein kinase (Kinghorn, 2001). Methi contains a high protein fodder, which provides hair with the necessary protein nutrition (Evans, 2009)<sup>[16]</sup>. The current study's findings could be attributed to the health-promoting properties of these plants. The R3 combination of olive oil, egg yolk, and vitamin resulted in a significant increase in hair length. This increase began in week 2 and lasted through till end of the study. When compared to plant extracts, the ombination of olive oil to yolk and vitamin E was considerably more effective. Vitamin E is made up of fat-soluble compounds called tocopherols and tocotrienols, which scavenge free radicals by scavenging peroxy radicals (Serbinova *et al.*, 1991)<sup>[17]</sup>. In patients with hair loss, eight months of diets supplemented with 50 mg of mixture tocotrienol and 23 IU of -tocopherol resulted in a 34.5 percent increase in hair



count, compared to a 0.1 percent decrease with placebo. The derivatives' potential to suppress lipid peroxidation could limit oxidative stress in hair follicles, trying to prevent hair loss; however, more research is required (Beoy *et al.*, 2010) [18]. Lysine is an essential amino acid discovered in meat and eggs that is thought to aid in iron absorption. After 6 months of diets supplemented with L-lysine (1.5 g), iron (72 mg), vitamin B12, vitamin C, biotin, and selenium in patients with chronic TE, there was a significant 39 percent reduction in hair shedding, as well as a substantial elevated serum ferritin levels in females who had previously failed with iron supplementation alone (Rushton *et al.*, 2002). Correspondingly, coconut oils were used for centuries to dress hair, particularly in the Caribbean Indo-Pak subcontinent and have been claimed to promote hair growth (Nadkarni, 1954; Hakim, 1997 [20]). These findings are in accordance with our results.

**Table 2:** Various treatments on hair growth in rabbits

	R1	R2	R3
Week 1	0.1+0.01	0.51+0.01*	0.4+0.02
Week2	0.6+0.01	0.92+0.01	0.100.02*
Week 3	1.23+0.03	1.64+0.03	1.53+0.04
Week 4	1.52+0.03	1.94+0.02	1.99+0.03**
Week 5	1.57+0.01	2.31+0.08*	2.42+0.058*
Week 6	1.76+0.02	2.51+0.05**	2.59+0.04*
Week 7	1.93+0.03	2.59+0.03**	2.63+0.05*

Values are expressed as Mean  $\pm$  SEM

\*, \*\* and \*\*\* represents significant variance at P-value < 0.05, 0.01 and 0.001 respectively

These vitamin E application results are consistent with Kamimura and Sasaki's findings (1964). The quantitative

effect of vitamin E in conjunction with coconut oil and egg yolk on hair growth appears to be similar to previous findings.

The radius of hair was measured using a microscope at various time intervals. It was discovered that coconut oil, vitamin E, and egg yolk increased hair diameter in rabbits only on the seventh week of treatment; however, the increase in hair widths was not statistically significant in the other weeks when compared to the control. Similarly, R<sub>1</sub> showed similar effects, and the size was observed to be highly larger in the seventh week when compare to control.

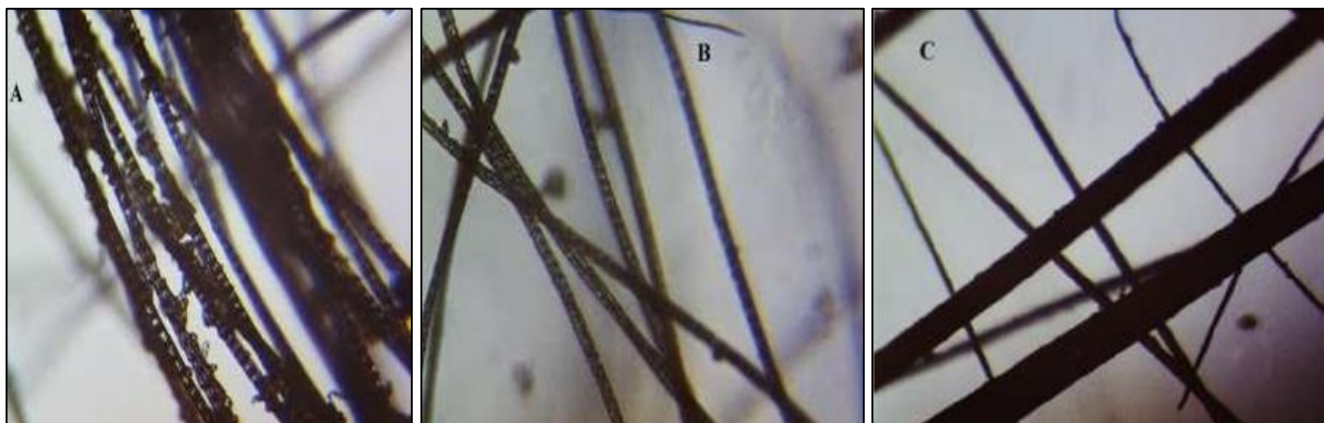
**Table 3:** Effect of different treatments on hair diameter in rabbits

	R1	R2	R3
Week 1	12+0.12	14.22+1.22	14.32+0.71
Week2	11.99+1.11	13.90+1.22	13.12+0.82
Week 3	13.12+1.03	13.34+0.78	14.22+1.03
Week 4	12.24+1.13	14.29+0.35	14.23+0.82
Week 5	13.72+1.05	13.24+0.58	14.21+0.52
Week 6	12.03+1.12	14.19+1.04	13.23+0.68
Week 7	13.11+1.03	13.23+0.75*	14.92+0.67**

Values are expressed as Mean  $\pm$  SEM

\*, \*\* and \*\*\* represents significant difference at P value < 0.05, 0.01 and 0.001 respectively

Hair health was determined by microscopy study of hairs. In control group, focal swelling, breakage of cuticle, and breakage of shaft was prominent (Figure 2). R1 and R2 showed reduced focal swellings in hair shafts, less number of split cuticles, and reduced broken ends of the hair shaft suggesting that these agents can be of worth importance for the hair health and needs to be further evaluated for its use in improvement of hair growth and health.



**Fig 2:** Photomicrograph of hair in control and treated groups (40 X). (A): slight focal swelling, breakage of cuticle, and breakage of shaft in control group. B: reduced focal swellings in hair shafts, less number of split cuticles, and reduced broken ends of the hair shaft

These data suggest that the applications of vegetable oils do accelerate hair growth but to varying degrees. This observation appears, at least partially to support the common Indo-Pakistani folk belief that certain oils promote man's hair growth. In addition, the ancient empirical use of these oils especially that of Sarson in daily dressing of the hair as mentioned by Nadkarni (1954) and Saeed (1969) appears to be justified. The oils of Amla and Coconut have been observed to exert hair growth promoting effect of short duration (from 2 to 4 weeks). Vitamin E as already mentioned has been suggested to promote hair growth in rabbits by improving local circulation and consequently the glucose and oxygen supply to the hair follicles. As a result, it is possible that vegetable oils tested contained varying

amounts of the fat-soluble vitamin E, which is abundant in wheat grain germ oil and many foods. This effect, however, could be attributable to the oils themselves, as fats have long been thought to cause bulging of fat layers of skin as well as active hair growth. This thickening could be caused by hair follicles growing downward into lipid layers (Ryder, 1958).

#### 4. Conclusion

The quantitative differences between the oils tested indicate that these oils may contain a hair growth promoter, most likely vitamin E or another substance. As a result, it is proposed that extensive research be investigated to assess the mode of action of these oils.

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