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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

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Abstract: Most people have little awareness about how to appropriately care for their hair and scalp. Healthy hair and healthy scalp typically complement each other, so a healthy scalp is needed to give the appearance of healthy hair and vice versa. Genetic and hormonal changes are important factors in hair loss, but weathering and grooming habits take a toll on our hair as well. The present study was designed to investigate the effect of the hair growth promoting effects of oil extracted from Amla, rittha and sikha Kai and effect of coconut oil mixed 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 T1. While in the third group, Coconut oil, Egg yolk and Vitamin E formulation was applied and was name T2. 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 daily topical applications of plant extracted oil have promoted the rabbit's hair growth ($P<0.05$) from the untreated control at the end of 1st week which continued till 7th week till the end of the treatment. Mixture of coconut oil, egg yolk and vitamin caused significant increase in the length of hair. This increase started from week 2 and continued till the end of the experiment. The combination of coconut oil with yolk and vitamin E was relatively more effective as compared to the plant extracts. The results also showed significant increase in diameter of hair which were exposed to coconut oil, vitamin E and egg yolk. 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 analyzed.

Key Words: Tropical, Application, Oils, Medicinal, Plants, Coconut Oil, Egg Yolk, Vitamin E , Rabbit Hairs and Karak

1. Introduction

Although healthy scalp and hair are of prime importance, the general public is not knowledgeable about these issues. Most people have little awareness about how to appropriately care for their hair and scalp. Healthy hair and healthy scalp typically complement each other, so a healthy scalp is needed to give the appearance of healthy hair and vice versa. A healthy scalp is generally defined by a lack of disease or abnormalities; however a person could be bald and perceived as having a healthy scalp. Experts in the field of dermatology (Rogaine®, 2009; Monselise et al., 2017) agree that the characteristics of healthy hair include thickness, volume, luster and resilience of the fiber. The majority

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have agreed that a healthy scalp would have normal follicular density with no scaling, itching, burning or erythema. Many patients experiencing hair loss assume the problem is related. They believe that treatment of the scalp would promote futurehair growth, although this is frequently not the case.

Hair growth is cyclic, with phases of growth (anagen), involution (catagen), and rest (telogen) (Uno, 1986; Montagna, 2012; Montagna and Ellis, 2013). The cycles of active growth and rest are regulated by complex messages between the epithelium and the dermis that are not yet well understood. In a normal scalp, most follicles are growing (90 to 95 percent), a few are undergoing involution (less than 1 percent), and the remainder are resting (5 to 10 percent)(Headington, 1993; Whiting, 1996; Blume-Peytavi et al., 2008). At the end of telogen, hair is released and shed and the next cycle is initiated. Each day, up to 100 hairs in telogen are shed from the head and about the same number of follicles enter anagen. The duration of anagen determines the length of hair, and the volume of the hair bulb determines the diameter. We are born with all our terminal hair follicles — approximately 100,000 on the scalp — that are predetermined to grow long, thick hair. Other follicles are predetermined to grow vellus hair, which is short, fine, and relatively nonpigmented and covers much of the body. Follicles can become larger or smaller under systemic and local influences that alter the duration of anagen and the volume of the hair matrix.

Androgenetic alopecia is hereditary thinning of the hair induced by androgens in genetically susceptible men and women (Hamilton, 1942; Otberg et al., 2007). This condition is also known as male-pattern hair loss or common baldness in men and as female-pattern hair loss in women. Thinning of the hair usually begins between the ages of 12 and 40 years in both sexes, and approximately half the population expresses this trait to some degree before the age of 50 (Hamilton, 1951; Piraccini and Alessandrini, 2014). The pattern of inheritance is polygenic (Küster and Happle, 1984). Pathophysiology in susceptible hair follicles of the scalp, dihydrotestosterone binds to the androgen receptor, and the hormone-receptor complex then activates the genes responsible for the gradual transformation of large, terminal follicles to miniaturized follicles (Messenger and Sinclair, 2006; Yazdabadi et al., 2008). With successive hair cycles, the duration of anagen shortens and the follicles become smaller, producing shorter, finer hairs that cover the scalp poorly. These miniaturized hairs of various lengths and diameters are the hallmark of androgenetic alopecia (Hamilton, 1951; Otberg et al., 2007). Hair suffers aggression; there can be some aliments to normal health of hair and cause trouble. The main problems associated with hair such as pigmentation problems (Fading), and falling of hair (Shedding). Synthetic drug, minoxidil is a potent vasodilator appears safe for long- term treatment.

After five years use of 2 and 3% topical minoxidil, the improvement has been shown to peak at one year with a slow decline in regrowth over subsequent years. Long-term treatment with local side effects may be a problem with continuing used of minoxidil lotion (Wilson et al., 1991; Bhalerao and Solanki, 2002). On the basis of market survey carried out on crude drugs used presently for herbal hair oils gives us clue for selection of drugs for hair oil. Hence the present study was aimed to evaluate the hair growth activity of herbal formulations, which includes oil extract of all mentioned drugs in various concentrations.

A plethora of herbs have been employed for hair treatments. A few of these herbs are amla, henna, neem, methi, lemon, tulsi, brahmi, shikakai, reetha, liquorice root, musk root, mahabhringraj, jantamasi, chitraka, marigold, hibiscus, nutmeg, parsley, rosemary, thyme (Poucher, 2013). Synthetic drug, minoxidil is a potent vasodilator appears safe for long-term treatment. After five years use of 2 and 3% topical minoxidil, the improvement has been shown to peak at one year with a slow decline in regrowth over subsequent years (Olsen et al., 2004). Long-term treatment with local side effects may be a problem with continuing used of minoxidil lotion (Wilson et al., 1991). On the basis of market survey carried out on crude drugs used presently for herbal hair oils gives us clue for selection of drugs for hair oil. Hence the present study was aimed to evaluate the hair growth activity of herbal formulations, which includes oil extract of all mentioned drugs in various concentrations. In order to justify the traditional claims now a days multi ingredient hair oils are prepared and tested for their hair growth activity.

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The Plant *Emblica officinalis* belongs to family Euphorbiaceae. Amla is rich source of vitamin C and contains appreciable amount of pectin rich in mineral matters like phosphorous, iron, and calcium (Wilson et al., 1991). *Bacopa* monnieri belongs to the family Scrophulariaceae, is known for its action on brains so that it is called as nervine tonic. It has been reported that bacosides A & B enhance protein kinase activity and new protein synthesis in cells. So that it is also used for treatment of dementia (Wagner and Bladt, 1996). *Trigonella foenum-graecum* belongs to family Leguminosae used as high protein fodder and for its cleansing and softening activity. It promotes scalp health and prevents hair falling (Adhirajan et al., 2003). Leaves of *Murraya koenigii* belongs to family Rutaceaceae is used for its antiseptic properties(Kumar and Jayaveera, 2014). *Cocus nucifera* from the family Palmae is said to promote the growth of hair.

Amla is rich in vitamin C, tannins and minerals such as phosphorus, iron and calcium which provides nutrition to hair and also causes darkening of hair (Wagner and Bladt, 1996). Hibiscus consists of calcium, phosphorus, iron, vitamin B1, riboflavin, niacin and vitamin C, used to stimulate thicker hair growth and prevents premature graying of hair (Monselise et al., 2017). Brahmi contains alkaloids which enhance protein kinase activity (Otberg et al., 2007). Methi contains high protein fodder which supply required protein nutrition to hair (Evans, 2009).

2. Materials And Methods

2.1. Study Animals

Five healthy adult 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 Zoology, Government Post Graduate College Karak, KPK, Pakistan. Animals were fed with rabbit diet and fodder and water was available *ad labitum*. Animals 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, University of Science and Technology, Bannu, KPK, Pakistan. The formulation is as under.

2.3. Plant Material

Seeds of gooseberry (Phyllanthus emblica), fruit of Sapindus mukorossi, also known as Reetha, and raw material of Senegalia rugata, commonly known in India as Shikakai were procured from local market and identified by comparing with standard herbarium specimens available in Department of boatany UST Bannu.The various parts of plant drugs are crushed in mixer and passed through the sieve number 80.

2.4. Preparation of oil

Direct boiling method was used to obtain the oil in which the crude drugs were powdered, weighed and directly boiled in olive oil with continuous stirring and heating until the drug had completely extracted in the oil base. The coconut oil was purchased from local market and was mixed with egg yolk 1:1 ratio and then vitamin E 40 mg/100 ml was added to the mixture. The oils were placed in clean glass bottles and were used 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 forthis 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

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

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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.

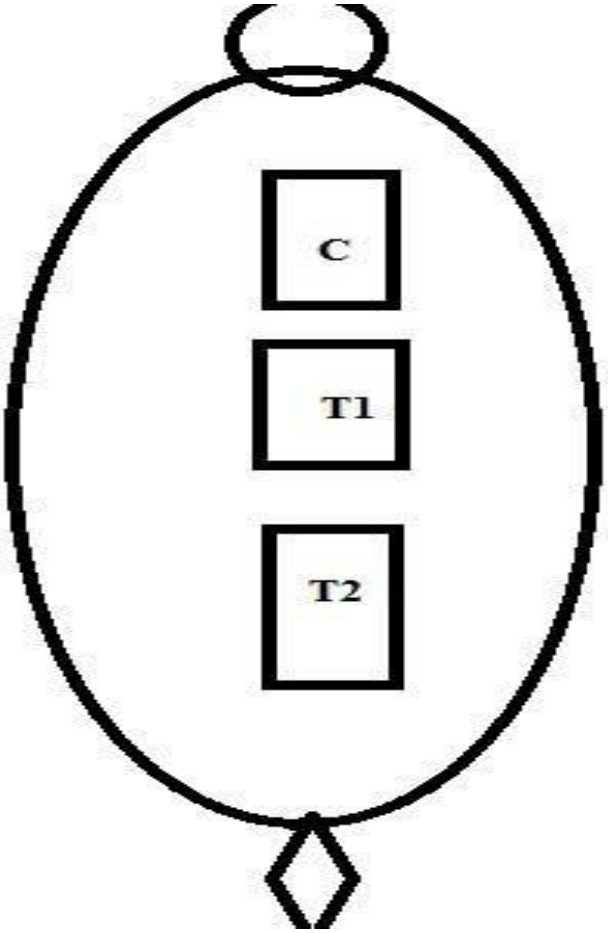

Figure 1: Spots selected for different treatments along the vertebral column of rabbit.

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

Results were represented as Mean ± SEM. One way analysis of variance followed by tukey's test was applied for statistical comparison of the results. P value less than 0.05 was considered significant.

3. Results And Discussion

Hair growth has been demonstrated to depend on the mitotic activity of hair bulbs in their follicles (Montagna and Ellis, 2013). Thus the substances enhancing absorption of glucose and Oxygen and in this way increasing the energy supply to the hair bulbs have been observed to stimulate the growth of hair. Similarly, local application of vitamin C which improves circulation around the hair bulbs has been reported to promote hair growth in rabbits (Kamimura and Sasaki, 1965) . The rabbit's hair growth was also found to be effected by sun-light, environmental temperature, feed and age. Moreover, to some extent hair growth is irregular and varies within individuals. However, when the animals are kept on similar managemental and environmental conditions and the sites of application

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are made on both sides of the vertebral column with changing positions, 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 irritation	NIL	NIL	NIL
Skin irritation	NIL	NIL	NIL

The daily topical applications of plant extracted oil have promoted the rabbit's hair growth ($P<0.05$) from the untreated control at the end of 1st week which continued till 7th week till the end of the treatment. Hibiscus consists of calcium, phosphorus, iron, vitamin B_1, riboflavin, niacin and vitamin C, used to stimulate thicker hair growth and prevents premature graying of hair (Adhirajan et al., 2003). Brahmi contains alkaloids which enhance protein kinase activity (Kinghorn, 2001). Methi contains high protein fodder which supply required protein nutrition to hair (Evans, 2009). The results of the present study might be due to the health promoting effects of these plants.

In the R3, mixture of coconut oil, egg yolk and vitamin caused significant increase in the length of hair. This increase started from week 2 and continued till the end of the experiment . The ombination of coconut oil with yolk and vitamin E was relatively more effective as compared to the plant extracts. Vitamin E consists of fat-soluble compounds known as tocopherols and tocotrienols that function as antioxidants by scavenging peroxyl radicals (Serbinova et al., 1991). Eight months of supplementation with 50 mg of mixed tocotrienols and 23 IU of α-tocopherol resulted in 34.5% increased hair count in 38 patients with hair loss, compared to a 0.1% decrease with placebo. The ability of the derivatives to inhibit lipid peroxidation may limit hair follicle oxidative stress, thus preventing hair loss; however, additional studies are needed (Beoy et al.,2010). Lysine is an essential amino acid found in meat and eggs, and is thought to play a role in the absorption of iron. In patients with chronic TE, supplementation with L-lysine (1.5 g), iron (72 mg), vitamin B12, vitamin C, biotin, and selenium resulted in a significant 39% reduction in hair shedding after 6 months, as well as a significant increase in serum ferritin levels in women who had previously failed with iron supplementation alone (Rushton et al., 2002). Similarly, coconut oils have been used for centuries for the dressing of hair especially in the Indo-Pak subcontinent and have been claimed to promote hair growth (Nadkarni, 1954; Hakim, 1997). These findings arein accordance with our results.

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Table 2: Effect of different 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	0.10±0.02*
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 ± SEM

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

These results of vitamin E application are in agreement with those of Kamimura and Sasaki (1964). Quantitatively, the hair growth promoting effect of vitamin E in combination with coconut oil and egg yolk appears to be similar to the previous findings.

The diameter of hair in different time intervals were determined through microscope. It was observed that coconut oil vitamin E and egg yolk increased the diameter of the hair in rabbits only on 7th week of the treatment however in other weeks the increase in hair diameters were not statistically significant compared to the control. Similarly, R1 exhibited similar effects and the diameter was found significantly high in 7th week as compared to the 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 ± SEM

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

Hear 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.

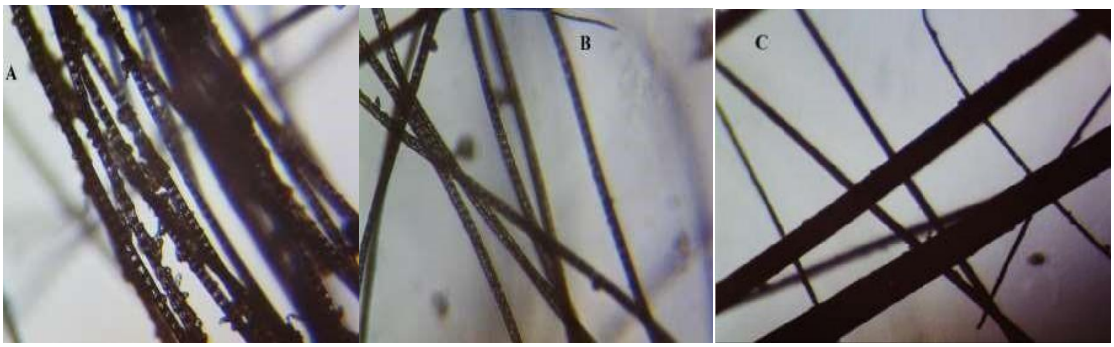

Figure 2: Photomicrograph of hair in control and treated groups (40 X). (A): slight focal swelling, breakage of cuticle, and breakage of shaft in control group. B: reduced focal swellings in hair shafts, less number of split cuticles, and reduced broken ends of the hairshaft.

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. It is, therefore, conceivable that the vegetable oils tested might be containing different quantities of the fat soluble vitamin E which is abundantly present in wheat germ oil and in many foods. However, this effect may be due to the oils themselves as the fats have long been suggested to cause thickening of fatty layers of skin along-with active growth of hair. This thickening might be due to downward growth of the hair follicles into fatty layers (Ryder, 1958).

4. Conclusion

The quantitative differences among various oils tested suggest that these oils may contain some hair growth promoter probably vitamin E or some other substance. It is, therefore, proposed that elaborate studies should be undertaken to determine the mechanism of action of these oils.

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