The Effects of Traditional Kurdistan Plant Extracts on Rat Hair Growth in vivo

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Abstract- Pathologies of hair growth can be psychologically distressing but they are poorly controlled. Hormones and paracrine factors regulate the hair follicle and its associated glands. However, our understanding of their mechanisms is limited, restricting the development of new treatments for hair disorders. Therefore better treatments for hair loss disorders are required. Some plant extracts are believed to have effect on hair growth. Few local plants in Kurdistan region are used traditionally as stimulators of human hair growth, but their effects on hair growth *in vivo* has not been studied yet. Therefore the aim of this study was to investigate the actual effects of those local plant extracts used as a traditional herbal treatment for hair loss, using *in vivo* rat model; and to compare their effectiveness with the best medical treatment available (Minoxidil).
The effects of extracts from Myrtus (*Myrtus communis*), Galls (*Quercus infectoria*), Oak (*Quercus aegilops*) both separately and mixed at the recommended concentrations were compared with the medical treatment for hair loss (Minoxidil) and a negative control group. Shaved backs of Wistar rats (6 weeks old) were treated daily for 42 days (six groups, n=6 per group), and the degree of their effectiveness was observed and compared with each other and with both positive and negative controls. Results show that the mixture of the three plants extracts and Minoxidil have similar significant hair growth promotion effect compared to other groups. Therefore, extracts from Myrtus, Galls and Oak (*Quercus aegilops*) stimulates rodent pelage follicles *in vivo* suggesting they can be used as promoter of hair growth in human.

Index Terms- Hair growth, *in vivo* study, plant extracts, Wistar rats.

I. INTRODUCTION

Hair is a key characteristic of mammals, and exerts a wide range of functions including physical protection, thermoregulation, sensory activity, and social interactions (Schneider et al., 2009). The HF is an attractive research model as hair growth, cycling and color are of profound interest to biological and medical researchers, and a vast industry that caters to individuals who wish to manipulate these parameters (Paus and Cotsarelis, 1999).The hair follicle (HF) consists of multiple different cell populations of neural crest, ectodermal or mesodermal origin, which are distinct in their location, function and gene and protein expression characteristics (Schneider et al., 2009, Paus and Cotsarelis, 1999). Additionally, the HF is a uniquely dynamic mini-organ that under-goes continuous cycling throughout adult life during which elements of its own morphogenesis are recapitulated (Fuchs et al., 2001).

In mammals, including human beings, hairs are cyclically shed and re-grown rapidly enabling it to renew itself and has the ability to cycle to produce a new hair and discard the old one (Panteleyev et al., 2001). During the hair follicle growth cycle, the upper portion of the hair follicle does not have a cyclic pattern, but the lower follicle does. The hair follicle growth cycle (Figure 1) has three stages. Anagen, or the growth phase, has different lengths depending on the area of the body and the species.

In catagen, the regression phase, the dermal papilla cells become inactive and the hair bulb becomes keratinised and moves up in the skin and forms a club hair, which rests in the hair follicle during telogen. At the end of telogen, or resting phase, the dermal papilla cells became reactivated and with related keratinocytes move back down into the dermis, forming a new hair bulb and lower follicle.

In exogenthe hair club fibre is shed from the follicle gradually (Higgins et al., 2009). The growth and unique cycling activities of the hair follicle is largely controlled by a group of specialized mesenchymal cells, located in a structure termed the dermal papilla (Higgins et al., 2013).

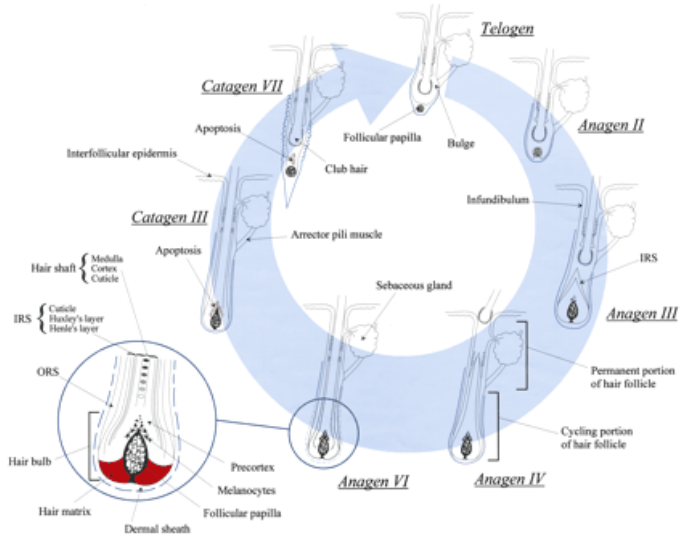

Figure 1: The mammalian hair cycle (Panteleyev et al., 2001).

Although pathologies of hair growth such as hair loss (alopecia) or excessive hair growth (hirsutism) are not physically painful or life threatening they do cause significant psychological distress. Several studies have reported the negative effects of hair loss in men and women (Girman et al., 1998, Cash, 1999, Girman et al., 1999). Hair disorders are poorly controlled due to the weak understanding of normal and abnormal hair follicle function and how established treatments work. The unsolved medical problems call for new approaches in developing effective remedies. This is therefore the focus of this study.

Minoxidil is the most preferable topical therapy for hair loss (Hordinsky, 2015, Vary, 2015) which works through ATP-sensitive potassium (K(ATP) channels in dermal papilla (Shorter *et al*., 2008). Traditionally extracts of local Myrtus (*Myrtus communis*), Galls (*Quercus infectoria*) and Oak (*Quercus aegilops*) have been used for promoting hair growth. The aim of this study is to investigate the effects of extracts from Myrtus, Galls of *Quercus infectoria* and Oak separately and mixed on rodent hair growth *in vivo*, compared with the most effective medical treatment available, Minoxidil.

II. Materials and Methods

This study was undertaken to examine the efficacy of local plant extracts for their potential role on hair growth by *in vivo* method. Male Wistar rats aged 6 weeks were randomly distributed into 6 groups to avoid any sibling bias and housed in groups of 6 with standard diet food pellets and water available at all time. Initially, the rats were partially anesthetized and dorsal hairs were removed externally by shaving (¬ 2cm width x 6 cm length) using an electric trimmer to reveal the pink skin. From the next day, termed day 0, each Wistar rat, except control group, was treated topically for 20 days with either the 5% extracts of Myrtus, Galls, Oakor combination of all 3 and Minoxidil (5%);

6 rats were used for each condition. The appropriate solution (¬100µl) was rubbed gently into the dorsal skin of each rat daily; new gloves were worn for each treatment type. Hair growth was recorded daily for each animal for 42 days, and dorsal photographs were taken at day 0, 7, 14, 21, 28, 35, and 42. The first day when visible hair growth could be seen was defined as the first day of anagen that subsequently increased and progressed to visible white hairs, was recorded for each animal. To calculate mean values, the first day of growth was designated as day 43 for any rat showing no hair growth signs by the last day. The day when the shaved dorsal area was fully covered with new hairs, i.e., there was no pink skin remaining and no visible difference in hair length to the adjacent unshaved areas, was also recorded.

III. Results

Generally the results show that topical treatment of 5% extracts of mixture from Myrtus, Galls and Oak promoted hair follicle growth similar to Minoxidil in rats *in vivo.*
The effects of local plant extracts on rodent pelage hair growth were assessed to determine whether those three plants which traditionally used for promoting hair growth could stimulate rat hair follicles *in vivo*, particularly when supplied through the skin directly, in comparison with negative control and the most effective medical treatment available, Minoxidil (positive control).

After shaving (under partial anesthesia) the dorsal skin of all rats remained pink, confirming that the hair follicles were in telogen (Figure 2). Negative control animals which didn't receive any treatment as the plant extracts were dissolved in distilled water, showed few skin changes until about day 28. In contrast, the extracts of mixture from Myrtus, Galls and Oak, as well as Minoxidil application for 20 days significantly ($P<0.001$) advanced the next hair cycle at 5% concentration, with new hair growth clearly visible around day 14 in most of the rats (Figure 3). The first sign of anagen occurred at 29.0 ±2.11 day (mean±SE) in negative control animals, while 5% Myrtus extract brought this forward to 21.3±1.15 day ($P>0.001$), 5% Galls extracts to 20.5±2.3 day ($P>0.001$), 5% Oak extracts to 23.2±2.87 day ($P>0.001$) and both 5% mixture (Myrtus, Galls and Oak) and Minoxidil even earlier to 13.1±2.2 day and 13±1.7 day respectively ($P<0.001$; mean±SE) (Figure 3A). This advancement of anagen extended across the dorsal area, despite treatment stopping after 21 days. Although 18% of negative control rats had completed anagen in all their dorsal follicles after 6 weeks, but the 5% mixture (Myrtus, Galls and Oak) and Minoxidil clearly exhibited anagen over the whole area, and this increased to 77 and 80%. Overall, 5% mixture (Myrtus, Galls and Oak) extracts and Minoxidil had significant effects on promoting hair growth in rats *in vivo*, with little difference between them (Figure 3B). The Myrtus, Galls and Oak extracts alone stimulated more follicle in to growth phase but their effect was much less than when combined (mixture).

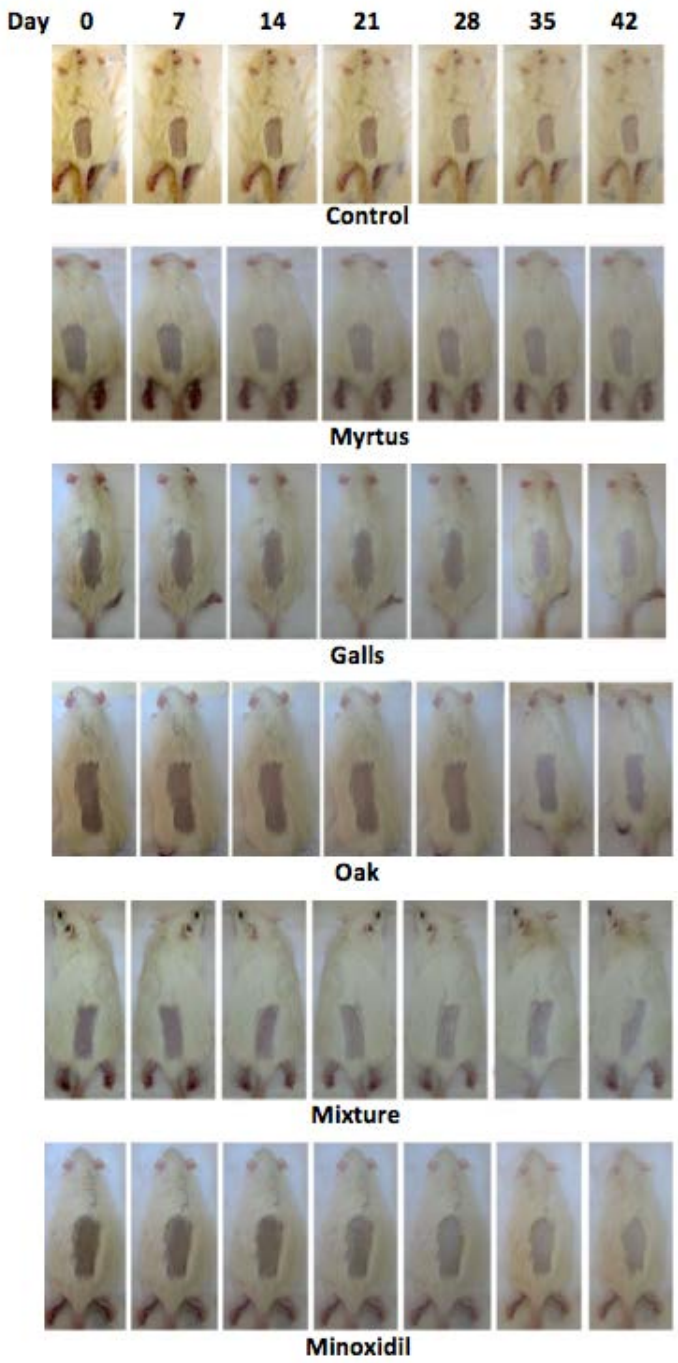

Figure 2. Topical application of 5% extracts of Myrtus, Galls, Oak, mixture of all three plants and Minoxidil promoted hair growth in rats *in vivo*. The treatments at 5% were applied topically to male rats for 20 days stimulated resting (telogen) follicles to start to grow and enter the next hair growth cycle (anagen). Pink shaved skin on back of the rats which was visible in day 0 photographs confirms telogen status of the hair follicles. Growing hairs are initially seen as whitening skin until white hair projects outside the skin (as seen in sequential photographs of individual animals). Toward the end of the experiment, 5% extracts of mixture (Myrtus, Galls and Oak) and Minoxidil had caused the most amount of hair growth in the rats compared to control.

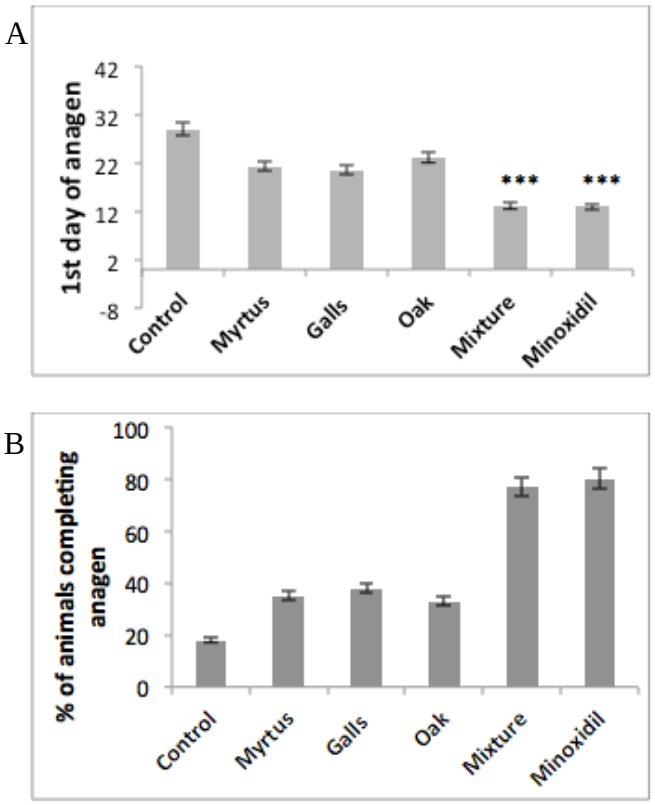

Figure 3. The topical application of 5% extracts of mixture (Myrtus, Galls and Oak) and Minoxidil significantly ($P<0.001$) advanced the first day at which anagen was visible, compared to the control alone (graph A) and increased the percentage of animals in which all follicles on the back had grown at day 42 (graph B). n=6 rats/treatment. ***$p<0.001$ vs. control.

IV. Discussion

Extracts of local plants have been used traditionally to promote hair growth, as it was believed that those plants extracts stimulate hair growth. Among the plants commonly used as herbal remedies for hair loss are local species of Myrtus (*Myrtus communis*), Galls (*Quercus infectoria*) and Oak (*Quercus aegilops*). However none of those local plant extracts were tested scientifically. Although many plant extracts have been tested for their antimicrobial activity, but very few were studied for their hair growth promoting effect (Erdogrul, 2002). An ethnobotanical survey was carried out in the Hawraman region, southern Kurdistan, Iraq, identified these plant species in the region and reported that these plants are currently utilized in various traditional uses, including medicines (Ahmad and Askari, 2015).

Male pattern hair loss or androgenetic alopecia directly distresses self-confidence affecting the individual's quality of life (Girman et al., 1998). Hair loss is therefore a significant psychosocial manifestation that worth much

expense on treatment. Androgenetic alopecia is noticed as a slow transformation of large scalp terminal hair follicles to shorter, thinner, and less deep vellus hair with a much shorter anagen (Randall, 1994). Although minoxidil, finasteride, and dutasteride including other synthetic therapeutic agents are mostly used for alopecia treatment, their adverse effects encourage sorting of alternative efficient treatment agent with a limited side effect particularly herbs (Lourith and Kanlayavattanakul, 2013).

Therefore the aim of this study was to investigate the effects of local plant extracts (separately and mixed) used by people as a traditional herbal treatment for hair loss, on rodent pelage hair *in vivo*, in comparison with negative control and the most effective medical treatment available, Minoxidil (positive control). The results of this study show that topical treatment of 5% extracts of mixture from Myrtus, Galls and Oak promoted hair follicle growth similar to Minoxidil in rats *in vivo.*

The shaved dorsal skin of all rats on day 0 showed pink color confirming that the hair follicles were in telogen (Figure 2). Negative control animals which didn't receive any treatment showed few skin changes until about day 28. In contrast, the 5% extracts of mixture from Myrtus, Galls and Oak, as well as Minoxidil application for 20 days significantly ($P<0.001$) advanced the next hair cycle at, with new hair growth clearly visible around day 14 in most of the rats (Figure 3 A). This advancement of anagen extended across the dorsal area, despite treatment stopping after 21 days. Only 18% of negative control rats completed anagen in all their dorsal follicles after 6 weeks, but the 5% mixture (Myrtus, Galls and Oak) and Minoxidil clearly exhibited anagen over the whole area, and this increased to 77 and 80% (Figure 3 B). Indicating that mixture of Myrtus, Galls and Oak extracts at 5% can stimulate hair growth *in vivo.*

Myrtus communis of the Mediterranean has been shown to have anti-inflamatory effect and also reported to influence hair growth (Chalchat et al., 1998). Various parts of this herb such as its berries, leaves and fruits have been used extensively as a folk medicine for several centuries. The herb is used traditionally for the treatment of disorders such as peptic ulcer, diarrhea, inflammation, hemorrhoid, pulmonary and skin diseases, although clinical and experimental studies suggest that it possesses a broader spectrum of pharmacological and therapeutic effects such as antioxidative, anticancer, anti-diabetic, antiviral, antibacterial, antifungal, hepatoprotective and neuroprotective activity (Alipour et al., 2014). However, not much of its effects reported on hair growth.

Myrtus communis Linn. or common Myrtle (Family - Myrtaceae) is one of the important drugs being used in Unani System of Medicine since ancient Greece period. It is recognized as Aas and its berries are known by the name of Habb-ul-Aas. It is often grown for its attractive foliage, flowers and berries. Its berries, leaves as well as essential oil are frequently used for various disorders (Sumbul et al., 2011).

Galls (*Quercus infectoria)* or (Aleppo oak) is a species of oak, bearing galls that have been traditionally used for centuries in Asia medicinally. Extracts of local Galls of *Quercus infectoria* is another herbal remedy traditionally used as a promoter of hair growth, antibacterial and antifungal (Digrak et al., 1999) and for helping wound healing (Jayaprasad et al., 2012). A study by (Sharma et al., 2011) observed new hairs on mice treated with *Quercus infectoria.*

Several studies carried out for determination of the effects of different plants extracts in animal studies, using rodents (Sharma et al., 2011). In search of natural extracts for hair growth, a study found that the extract of dried root of Sophoraflavescens has outstanding hair growth promoting effect. After topical application of Sophoraflavescens extract onto the back of C57BL/6 mice, the earlier conversion of telogen-to-anagen was induced (Roh et al., 2002).

Another study was undertaken to examine the efficacy of essential oil from seeds of Zizyphus jujuba for its potential role on hair growth by *in vivo* animal study method, in which essential oil was applied at different concentrations over the shaved skin onto the backs of BALB/c mice and monitored for 21 days. After 21 days, mice treated with oil produced a greater effect on the length of hair compared to the control (Yoon et al., 2010).

Overall, 5% mixture of local Myrtus, Galls and Oak extracts and Minoxidil had significant effects on promoting hair growth in rats *in vivo*, with little difference between them. Suggesting that their effects on hair growth may be mediated through the regulation of growth factors in dermal papilla cells. Further analysis of these plant extracts may lead to the development of new hair growing products.

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