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A Novel Therapeutic Lotion For Treating Alopecia Using Medicinal Plant Extracts and its Effects on Human Hormone Profiles

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ABSTRACT

Objectives: To examine the action of a novel composite lotion of medicinal plants extract for treatment of Alopecia and to outline its effects on human hormones profile. Design and Methods: Eighty four volunteers were enrolled in the prospective study. The new composite lotion was prepared by mixing the following components: Polar solvent extract of medicinal herb, Polypeptide compounds including keratin and keratin derivatives, and Methionine. These components were mixed in a ratio 5: 0.5: 0.1; respectively. All patients were advised to use the new composite lotion to be applied on the scalp skin once, twice daily or every other day for a period of four to six months. Moreover, hormones levels were measured using ELISA procedure. Results: After treatment, revealed cessation of hair loss while starting stimulation of inactive hair follicle and hence, hair growth. Moreover, the current study resulted that the novel product has no effects on hormones profile. Conclusion: These pharmaceutical achievements may be a result of stimulation of an inactive hair follicle or modulating the dysfunction of the hair follicles or by improving the general physiological status of the scalp skin while no any drawback was observed for the hormones profile.

Key words: Alopecia – Hair Loss – Hair Follicle Stimulation – Hair Growth – Medicinal Plant Lotion.

Introduction

There are three main phases of hair growth cycle; anagen, catagen, and telogen phases. Anagen is the active growth phase when hair fiber is produced. This is followed by catagen, a period of controlled regression of the hair follicle. Ultimately, the hair follicle enters telogen phase where it is in a so-called resting state (Dry, 1926). Alopecia Areata (AA) is a medical disease in which hair is lost from some or all areas of the body, usually from the scalp. Alopecia can affect men, women, and children. AA is sometimes known as "Area Celsi" because it was first described by Cornelius Celsus in 30 AD. Celsus described two types of Alopecia. The first was described as complete baldness occurring in people of all ages. The second was called Ophiasis, literally translated as "snake" due to the progressive way of the bald region which spreads across the skin. He suggested that Ophiasis was only seen in children (Emad, 1999). Because it causes bald spots on the scalp, especially in the first stages, it is sometimes called spot baldness. This condition may spread to the entire scalp (Alopecia Totalis) or to the entire epidermis (Alopecia Universalis) (Olivetti and Bubola, 1965).

Alopecia universalis is a medical condition involving rapid loss of all hair, including eyebrows and eyelashes. It is the most severe form of AA, with an incidence of 0.001% (1 in 100,000). Alopecia Universalis may occur at any age, and is currently believed to be an autoimmune disorder (Odom, et al., 2006). There is no standard treatment for Alopecia Universalis. Many treatments have been explored, including immunomodulatory agents such as imiquimod (Robins, 2007).

Alopecia Areata is not contagious. It occurs more frequently in people who are related to affected family members, suggesting that heredity may be a factor. Previous study identified at least four regions in the genome that are likely to contain AA genes (Letada, et al., 2007). German researchers identified the androgen receptor gene as the cardinal prerequisite for balding (Martinez et al., 2007). In the same year the results of this study were confirmed by other researchers (Hillmer and Hanneken, 2005). This gene is recessive and a female would need two X chromosomes with the defect to show typical male pattern alopecia. Seeing that androgens and their interaction with the androgen receptor are the cause of androgenic alopecia (AGA) it seems logical that the androgen receptor gene plays an important role in its development. Other genes involved with hair loss have been found, including a gene located at 3q26 (Levy-Nissinenbaum and Bar-Natan, 2006). This gene is also

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involved in a type of baldness associated with mental retardation. This gene is recessive. Another gene that might be involved in hair loss is the P2RY5. This gene is linked to hair structure. Certain variants may lead to baldness at birth while another variant causes "wooly hair" (Hillmer and Flaquer, 2005).

In addition, AA is widely regarded as an autoimmune disease where the anagen hair follicles apparently become the target for an immune cell attack. The condition is thought to be an autoimmune disorder in the body that attacks its own hair follicles and suppresses or stops hair growth. For example, T cell lymphocytes cluster around affected follicles causes inflammation and subsequent hair loss (Ranki, et al., 1984; Petukhova, et al., 2008).

Traumas such as, childbirth, major surgery, poisoning, and severe stress may cause a hair loss condition known as telogen effluvium (Perret, et al., 1984) in which a large number of hairs enter the resting phase at the same time, causing shedding and subsequent thinning. Some treatments used to cure mycotic infections can cause massive hair loss (Noruka and Nnoruka, 2005).

Oral corticosteroids dosage decrease the hair loss, but only for the period during which they are taken, and these drugs have serious adverse side effects and frequently fail to enter the skin deeply enough to affect the hair bulbs, which are the treatment target (Robins, 2007). In addition, some authors hypothesized that diphenylcyclopropenone has shown significant hair regrowth in 40% of patients with AA at 6 months, and being sustained in two thirds of these after 12-month-follow up-period (Pappas et al., 1995). Joly (2006) suggested that Methotrexate and corticosteroids are proposed treatments for Alopecia Totalis or Universalis (Sotiriadis, et al., 2006).

The principal objective of the present study is to examine the action of a novel composite lotion of medicinal plants extract for treatment of Alopecia Areata and Alopecia Totalis. This investigation prescribes a new topical treatment for Preventing Hair Loss and Baldness. Moreover, the important aim of the prospective study is to ensure that the novel natural seaweed lotion offers safety for all patients without causing any drawback for the hormones profile.

Design and Methods:

2.1. Patients:

Eighty four volunteers were enrolled in the prospective study; 68 Hair Loss patients and 16 healthy controls. They range from 12 to 60 years of age and all gave the informed consent. They were divided into two main groups: the first one was 50 Male group which included 7 individuals serving as a control, 14 individuals suffering from Alopecia Areata, 8 individuals suffering from Alopecia Totalis, 15 individuals suffering Hair Loss, and 6 individuals suffering from Baldness. The second group was 34 Female which included 9 individuals serving as a control, 11 individuals suffering from Alopecia Areata, 4 individuals suffering from Alopecia Totalis, and 10 individuals suffering Hair Loss. The study protocol and informed consent were approved by the Ethics Committee.

2.2. Lotion Composition:

Hair treatment compositions comprise (A), an extract obtained by polar solvent extraction of a plant, preferable types are Nigella Sativa, Birch and Rosemary and (B) a polypeptide compound including Keratin and Keratin derivative. Hair treatment composition can impart good hair style retentivity, good touch of feeling besides regrowing of the hair loss in case of Alopecia Areata, Alopecia Totalis and hair loss. We have made extensive studies on ingredients of many products, as a result, it was found that when a combination of a specific type of plant extract and a polypeptide is mixed with hair cosmetic composition such as shampoo, rinse, set lotion and hair spray so that the resulting composition can impart good hair style retentivity and a suitable degree of feeling to the touch beside good results of treatment. Accordingly, our lotion consisted of polar solvent extraction of a plant (A) and (B) a polypeptide compound. The (A) ingredient used in a previous study was obtained by extraction of plants. Examples of the plants included- Birch, Rosemary, Arnica, hamamelis, Camomile, Sage, St. John’s Bread, henna, hop, lime, Aloe, Wild thyme, Calendula, horsetail, Mountain Gentian, Nettle, Chestnut, Avocado, Milfoil, Coltsfoot, Marigold, Peach, Rose, Senna, Thyme and white lily.

Preferable parts of these preferable plants for the extraction are, for example, bark of birch, an entire grass part of rosemary, leaves of hamamelis, flower of chamomile, leaves of sage, leaves of aloe, leaves of henna and fruit of st. John’s bread. The plant extract is obtained by extracting leaves with solvent at room temperature or under heating conditions according to any knowthe traditional extraction technique. The extraction solvents are polar organic solvents including, for example, lower alcohols such as methanol, ethanol and the like, propylene glycol, 1,3- butylene glycol and glycerin and water. These solvents may be used singly or in combination (Novaron, 1987; Joly, 2006; Novarom, 2011).
2.3. **Lotion Concentrations and preparations:**

When the plant extract is in a liquid form, the ingredient (A) is used in an amount ranging from 0.001 to 10.0 wt%, preferably 0.01 to 1.0 % (hereinafter referred to simply as %) of the total composition, calculated as a residue obtained after evaporation of the extraction solvent there from. The ingredient (A) or plant extract maybe directly added to hair cosmetic compositions in the form of liquid extract, or maybe added after concentration to a desired level or after complete removal of the extraction solvent. The ingredient (B) used in the present study are polypeptide compounds which are, for example, keratin and decomposition derivatives thereof.

Keratin is derived from, for example, animal hairs, human hair, feathers, nails, horns, hooves, scales or the like. These materials maybe used in the form of fine powder and are preferably used as a decomposition derivative thereof. Preferable keratin materials are wool, human hair and feathers.

The decomposition derivatives of keratin materials are hydrolyzates, oxidative decomposition products, and SH group-modified compounds of reductive decomposition products. The hydrolysis techniques include an acid hydrolysis using hydrochloric acid, sulfuric acid, or phosphoric acid and alkali hydrolysis using sodium hydroxide, sodium carbonate and an enzyme hydrolysis using protease. The disulfide bond is split by reduction reaction into a thiol group. The chemical modification of the thiol group is conducted according to many investigators.

The lotion composition according to our study was prepared by adding the essential ingredients (A) and (B) to known ingredients of various kinds of seaweeds which vary according and depending on the type of the problem or disease of hair (Hair loss- alopecia (areata-totalis-universalis)-baldness. The lotion composition in this study for treatment was formulated in one form which is aqueous solution whereas the other forms like shampoo, hair rinses, hair conditioners, hair setting agents (lotions, sprays) and hair dyes are still under investigations (Novaron 1987; Joly 2006; Novarom 2011).

2.4. **Blood samples Collection and Storage:**

Five ml peripheral blood samples were withdrawn from all volunteers enrolled in this study by venipuncture. Blood samples were centrifuged at 2000g for serum separation. Serum was used for hormones investigations. The serum promptly was separated in a refrigerated centrifuge at 4°C and stored at -20 °C.

2.5. **Methods:**

Samples of blood were analyzed for Prolactin, FSH, LH, and Progesterone before treatment and at the end of treatment for the female group. Moreover, samples of blood were analyzed for Testosterone, Dihydrotestosterone, and Estradiol before and at the end of treatment for the male group. Blood samples were analyzed for estimation of the previous hormones using ELISA technique. All hormones will be determined quantitatively in serum using DRG kits supplied from (DRG International, Inc., USA ).

2.6. **Statistical Analyses:**

Results of the hormones profile were expressed as mean ± standard error (S.E.). Statistical analyses were performed using the student's test and chi-square test. Correlation was considered statistically significant at (P< 0.05).

3- **Results:**

3.1. **The Therapeutic Effect of the Lotion:**

The results are elucidated by colored photographs prior and after treatment with the new composite lotion and revealed cessation of hair loss while starting stimulation of inactive hair follicle and hence, hair growth in both Hair Loss and Baldness patients as shown in (Fig. 1), and in Alopecia Areata, and Alopecia Totalis as shown in (Fig. 2).
Fig. 1: This figure elucidated prior (A, C, and E figures) and after (B, D, and F figures) treatment with the new composite lotion in Hair Loss (A & B, and C & D figures) and Baldness (E & F figures) patients.

Fig. 2: This figure illustrated before (A, and C figures) and after (B, and D figures) therapy with the new composite lotion in Alopecia Areata (A & B figures) and Alopecia Totalis (C & D figures) patients.

3.2. The Impact of the Lotion on Hormones Profile:

The measurements for hormones profile for the male group before and after treatment (Table 1 & 2) revealed that Alopecia Areata, Alopecia Totalis, Hair Loss, and Baldness in male volunteers showed insignificant changes (P > 0.05) for Testosterone, Dihydrotestosterone, and Estradiol when comparing their serum levels before treatment with their serum concentrations at the end of treatment.

Alopecia Areata, Alopecia Totalis, and Hair Loss in female volunteers showed insignificant changes (P > 0.05) in Prolactin, FSH, LH, and Progesterone when comparing their serum levels before treatment with those at the end of treatment.

The current study hypothesized that the novel natural plants extract lotion offers safety for all patients without causing any drawback on the hormones profile.

<p>| Table 1: The measurements for hormones profile for male group before and after treatment with the new composite Lotion |</p>
<table>
<thead>
<tr>
<th>Hormonal Levels</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Testosterone (ng/ml), mean ± S.E.</td>
<td>4.33 ± 1.3</td>
<td>4.72 ± 1.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serum Dihydrotestosterone (mol/l), mean ± S.E.</td>
<td>0.75 ± 0.1</td>
<td>0.74 ± 0.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serum Estradiol (pg/ml), mean ± S.E.</td>
<td>33.0 ± 1.7</td>
<td>33.0 ± 1.0</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

(P >0.05): represents a non-significant difference.

<p>| Table 2: The measurements for hormones profile for female group before and after treatment with the new composite Lotion |</p>
<table>
<thead>
<tr>
<th>Hormonal Levels</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Prolactin (ng/ml), mean ± S.E.</td>
<td>14.0 ± 2.7</td>
<td>14.0 ± 1.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serum FSH (mIU/ml), mean ± S.E.</td>
<td>7.1 ± 1.3</td>
<td>7.0 ± 0.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serum LH (mIU/ml), mean ± S.E.</td>
<td>3.2 ± 1.8</td>
<td>3.1 ± 1.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Serum Progesterone (ng/ml), mean ± S.E.</td>
<td>0.19 ± 0.2</td>
<td>0.18 ± 0.4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

(P >0.05): represents a non-significant difference.

Discussion:

It has been reported that hair loss is caused when there is a partial or complete cleavage between the papilla and the hair bulb as a result of bacterial attack, deep stress, immunological disturbances, genetic, hormonal imbalance, dandruff, and diseases such as fever, diabetes, syphilis (Leon 1975; Stanley and Gilbert 1975). From previous studies on this broken link between the papilla and the hair bulb it has been found that such a link consists of some amino acids, vitamins, polypeptide chain and polysaccharides of high molecular weights. Therefore, throughout the current research work we were able to extract some effective extracts from certain...
types of natural medicinal plants and we found that their composition is compatible with that of the joint between the papilla and the hair bulb. These new extracts were mixed with polypeptide compounds (keratin and keratin derivatives) and Methionine and were applied as a lotion for the hair; the results revealed their effective role on two forms of Alopecia, namely: Alopecia Areata and Alopecia Totalis.

The results obtained were striking. All of the volunteers markedly responded to the action of the lotion where dense hair bundles started to grow on. The new lotion proved to be effective for treatment of the three different forms of hair loss and the results were high significant as compared with those people who used the most powerful lotion "Minoxidil" [22], which can only realize 20% response for those patients suffering from Alopecia Areata. Moreover, Administration of Minoxidil requires proper medical follow up for a period of not less than six months in order to alleviate any adverse side effects.

On the other hand, the new lotion is nothing but an extract from certain types of plants and which exerts no side effects and is quite safe when used. We suggested that this new lotion acts in coordination with hair follicle activation for stimulation of the hair bulb.

It has been reported that the male hormone testosterone and the steroid 5 alfa- reductase also play a principal role in hair growth [24]. Some authors suggested that treatment of prostate disorders by administration of a daily dose of 1 mg tablet of finasteride (propecia) for a long period of more than one year has a limited role in preventing hair loss, but it is not capable to stimulate hair growth (Sawaya and Price, 1997). Other authors suggested that in cases of high levels of 5 alfa-reductase, the male hormone testosterone is transferred to dihydrotestosterone which is usually followed by hair loss (Price,1987; Harris and Kozarich, 1997; Oh, et al., 1998).

Application of the new lotion of plant extract in the cases of Alopecia Totalis has resulted in appearance of new hair growth in the various parts of the body. The new composite lotion of medicinal plants extract for treatment of hair loss was prepared by mixing the following components: Polar solvent extract of medicinal herbs, Polypeptide compounds, and Methionine and is in agreement with other research works who use polar solvent extraction of birch, rosemary and hamamelis to give good hair style, retentively and a suitable degree of feeling to the touch. The novel lotion extract is also a good resource of vitamins and polysaccharides of high molecular weight (Boudou and Reygagne, 1997). The use of polypeptide compounds including keratin and keratin derivatives were used and that was supported with the use of sulphur-containing material (methionine) that has an average MW of 10,000 or less, that can form disulphide bonds involving the keratin of hair and whose sulphur content is 1% by weight (Tokyo and Yachiyo, 1986).

The use of methionine is desirable because it can contribute to the hair under different conditions and can supplement the loss of methionine and cystine from the keratin of the hair during hair loss (Burns, et al., 1986)

So this novel lotion is a composition for regulating and repair the damage of the hair as it comprises a safe and effective amount of a polypeptide having the structure of those derived from dermal papilla cells with characteristics pH ranging from 2- 3.5. The growing of hair of the patients by this lotion during the anagen phase is pharmaceutically logic. We can scientifically postulate that the hair loss may disturb the link between papilla and the hair pulp causing a damage varying from 10% to 100%. By using the new lotion, which comprises some amino acids, vitamins, polypeptide chains and polysaccharides of high Molecular weight, which pharmaceutically behave as active carriers, we can repair the damage caused in the link between papilla and the hair pulp.

Moreover, these pharmaceutical achievements may be a result of stimulation of an inactive hair follicle or modulating the dysfunction of the hair follicles or by improving the general physiological status of the scalp skin. This lotion plays a role in maturation and differentiation of those cells specifically involved in hair growth either directly or by stimulating the organs cells and activating biomaterials associated with the hair follicle. Besides, the hormones profile is not affected at the end of the treatment. So, we can suggest that the novel lotion has no side effect on hormones and is safe for humans.

Finally, we can summarize the mode of action of the present study novel lotion as it is a natural seaweed extracts that offers safety without any drawback on hormones profile It is provides the essential nutrients for the matrix and cortex cells and allowing them to proliferate and growing the hair. It is contains polysaccharide materials and vitamin "A" which promote restoring the link between the bulb and papilla, and it offers a properly adjusted pH lotion to combats dandruff.

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