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Targeting the real issues in men's care: Reducing hair loss and irritation after shaving

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Abstract Two big issues for many men are hair loss and rashes after shaving. Discovering cosmetic actives that help in treating these problems are important for developing men's grooming products. Here we show that an extract from the cactus *Opuntia ficus-indica* improves skin condition by reducing skin irritation after shaving. Furthermore, *in vitro* and *in vivo* studies show that hair loss caused by the hormone dihydrotestosterone can be reduced by scalp treatment with an extract from *Ocimum basilicum*.

INTRODUCTION

While some women's and men's skin care needs overlap, such as the necessity for moisturisation, there are some distinct issues that each gender has to face. For example, men are much less likely than women to develop cellulite due to a different cutaneous composition and connective tissue arrangement. On the other hand, men experience hair loss more often and to a greater degree and, for many men, a daily facial shaving routine leads to skin irritation. It is important to consider the specific physiology of male skin to target skin care for men. Here, we present two cosmetic actives that tackle these two issues that are prevalent in men.

Sensitive skin in men - exacerbated by shaving

More than 1 in 3 men feel that they have sensitive or very sensitive skin (1). Sensitive skin tends to be more susceptible to some environmental factors. People with sensitive skin report exaggerated reactions such as redness, itching or rashes when their skin is in contact with certain cosmetics, plants, fabrics, or temperature.

At a molecular level, an irritating stimulus on keratinocytes leads to a signalling cascade that activates sensory neurons in the skin, which in turn produce the Calcitonin Gene-Related Peptide (CGRP). This signalling molecule activates mast cells, which release histamines, leading to itching and inflammation (2) (Figure 1A).

Daily shaving of facial hair can contribute to skin sensitization as it stresses the skin by continuously removing the top layers of the stratum corneum. The skin barrier is further disrupted by detergents in shaving foam that remove protective lipids in the skin leading to burning, irritation and redness of the skin. Therefore, it is important that shaving products and

after shaving treatments contain cosmetic ingredients with a soothing effect.

A skin soothing active from a cactus

There are about 200-300 cactus species of the genus *Opuntia* that grow all over the world in arid and semi-arid zones. Commercial cultivation is carried out in Italy, Spain, Mexico, Brazil, Chile, Argentina and California. Traditionally, *Opuntia* cactus plants serve as sources for fruits and vegetables and for medicinal and cosmetic purposes. The dried powdered cactus leaves are a rich source of minerals, pectins and flavonoids (3). There are several scientific publications that report about an analgesic action and anti-inflammatory properties of *Opuntia* extract (4). This makes *Opuntia* an ideal candidate for a cosmetic ingredient to soothe irritated skin. Here we conducted *in vitro* and *in vivo* experiments to investigate the ability of *Opuntia ficus-indica* extract to soothe irritated skin.

Male pattern hair loss

The typical person has between 100,000 and 150,000 hairs on their head. In order to maintain a normal volume, hair must be replaced at the same rate at which it is lost, which amounts to about 100 strands of hair over the course of a day. If this number is clearly exceeded over a longer period of time, the individual is experiencing hair loss. The technical term for hair loss is alopecia and it can occur for a variety of reasons such as infections, nutritional deficiencies, stress, trauma, drugs, or autoimmune phenomena. The most common type of hair loss in men however, which affects up to 70% of men in their life, is androgenic alopecia. It involves gradual hair thinning, which often takes place over the course of several decades. It can start at any age, is progressive and hereditary.

The cause of androgenic alopecia is male hormone-related but it is not primarily caused by excessive testosterone. Instead, the hair follicles become more sensitive to the hormone due to a genetic predisposition. An enzyme, 5 α reductase, leads to the conversion of the male sex hormone testosterone to the active form dihydrotestosterone (DHT), which has two to three times greater affinity for androgenic receptors. DHT causes the hair follicles to produce thinner and smaller hairs up until the point that the hairs actually stop growing. Therefore, reducing the production of DHT by inhibiting 5 α reductase could decrease hair loss. Importantly, there are three isoforms of 5 α reductase (type I, II and III), with 5 α reductase type II being the isoform detected in different parts of scalp hair follicles (5) (6). This makes 5 α reductase II the main target for anti-hair loss products.

Fighting hair loss with the king of herbs

Basil is a common name for the culinary herb *Ocimum basilicum*. The word comes from the Greek word *basileus*, which means "King". Indeed, basil is considered to be the "King of Herbs" and plays an important role in both Italian and Asian cuisine. Basil contains high concentrations of essential oils and potent antioxidants with anti-ageing, anti-cancer, anti-viral and anti-microbial properties. Thanks to these valuable components, basil is also used for its medicinal properties in Ayurveda, the traditional medicinal system of India. We conducted *in vivo* and *in vivo* studies to assess the ability of a hairy roots extract of *Ocimum basilicum* (Basil hairy roots extract, see experimental section) to inhibit 5 α reductase type II activity and to reduce hair loss.

EXPERIMENTAL SECTION

Production of *Opuntia ficus-indica* extract

Dried leaves of the species *Opuntia ficus-indica* served as source material. The material was taken up in a phosphate buffer solution, digested with a special enzyme mixture and filtered by cross flow filtration through a 10 kDa membrane.

CGRP release in a neuron/keratinocyte coculture model

Sensory neurons were cultivated in 96 well plates in coculture medium. After 10 days, normal human keratinocytes were seeded in each well. After 2 days of coculture, supernatants were changed to coculture medium with or without 0.33% *opuntia ficus-indica* extract and cells were incubated for 30 minutes followed by stimulation with 10⁻⁶ M capsaicin for 20 min. The presence of CGRP in the supernatants was determined by ELISA. Treatment of the coculture with lidocaine at 10⁻⁶ M was used as positive control.

Clinical study to assess a soothing effect after shaving

In a double-blind, placebo controlled clinical study, 20 men (35 – 65 y) induced skin irritation by repeated shaving of their forearms with a commercial razor and shaving foam. Immediately afterwards, the irritated areas were treated with an emulsion containing 2% *opuntia ficus-indica* extract or the corresponding placebo emulsion. Skin redness was measured using a Chromameter CR 300 (Minolta) before and 30/60/120 min after shaving.

Production of basil hairy roots extract

To produce basil hairy roots extract, an *Ocimum basilicum* leaf was infected with the soil bacterium *Agrobacterium*

rhizogenes and cultivated on an agar plate. The transformed leaf cells then started to grow tiny roots – the so-called "hairy roots". The hairy root cultures were further propagated in specifically designed bioreactors. Roots were then harvested and extracted in an appropriate medium according to a standard protocol.

5 α reductase II inhibition assay

Cell extract from human embryonic kidney (HEK293) cells stably expressing 5 α reductase type II was added to the steroid hormone androstenedione in Tris HCl-EDTA-buffer containing 0.24 mM NADPH in the presence or absence of basil hairy roots extract. Treatment with Finasteride (7) served as a positive control. After incubation at 37°C for 30 min, reactions were stopped by the addition of NaOH. The reduction of androstenedione to 5 α -androstenedione was detected via liquid chromatography followed by mass spectrometry (LC-MS/MS; Surveyor MS Plus HPLC system connected to a TSQ Quantum Discovery Max triple quadrupole mass spectrometer, both from Thermo Fisher Scientific).

Clinical hair loss study

A mixed gender panel of 21 volunteers (25 – 67 y, average 51.1 y), who suffered from mild to moderate hair loss (hair loss > 100 hair strands per day) applied the test fluid containing 1 % basil hairy roots extract every day in the evening for two months. Volunteers collected their lost hair (mornings and evenings, only combed hairs in the brush) and placed them in prepared envelopes, which were collected and counted at the test institute. Hair collections were done on 3 consecutive days and the average of the total counts were taken: day 0 - 3 (baseline), on day 25 – 27 (1 month) and day 53 - 55 (2 months) respectively.

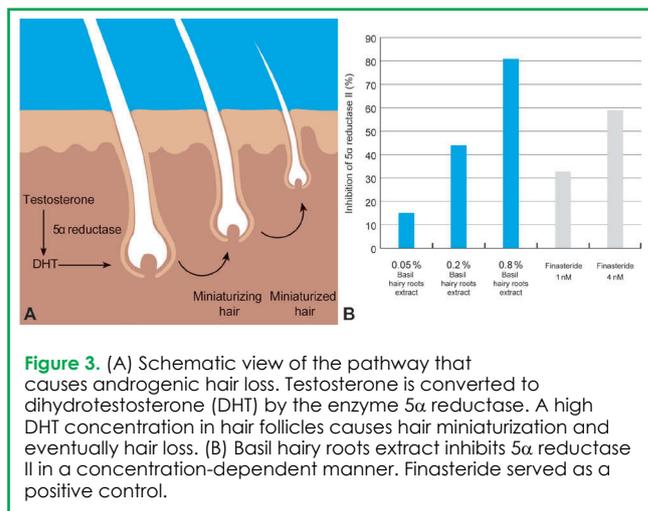
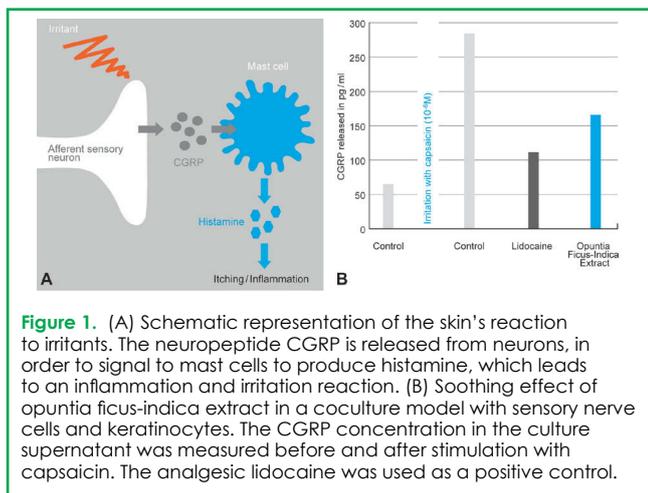
RESULTS AND DISCUSSION

Opuntia ficus-indica extract reduces the release of stress factors

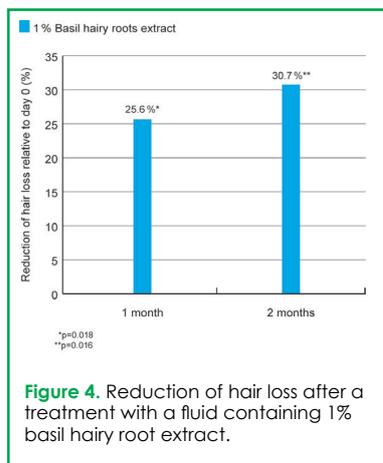
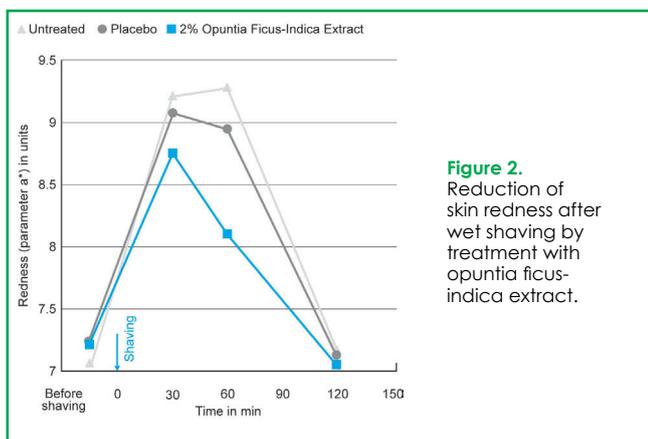
To investigate a potential soothing activity of *opuntia ficus-indica* extract, neurons in coculture with keratinocytes were stimulated with capsaicin, an irritant present in chili peppers, and the amount of released CGRP (Figure 1A) was measured as a marker for irritation. Addition of capsaicin increased CGRP levels in the medium more than fourfold, indicating an upregulated stress signalling by the cultured neurons. Lidocaine, a local anaesthetic that blocks the signal at the endings of sensory nerves, is used as positive control for CGRP reduction. In the presence of 0.33% *opuntia ficus-indica* extract, CGRP release was decreased by 42% compared to the untreated but capsaicin stimulated control (Figure 1B). This suggests that *opuntia ficus-indica* extract has a soothing effect on neurons in the skin.

Opuntia ficus-indica extract soothes skin after shaving

To demonstrate an immediate soothing effect of *opuntia ficus-indica* extract *in vivo*, a clinical study was carried out where a panel of 20 men irritated their skin with repeated wet shaving and afterwards applied an emulsion containing *opuntia ficus-indica* extract and the corresponding placebo emulsion. Skin redness was measured by Chromametry as a marker for skin irritation. Shaving led to a measurable increase in skin redness (Figure 2). Treatment with *opuntia ficus-indica* extract reduced redness of the skin, with a



statistically significant ($p < 0.05$) difference compared to placebo-treated skin 60 Minutes after shaving (Figure 2). This shows that after one single application, opuntia ficus-indica extract is able to decrease the effect of physical irritation on the skin.



month (Figure 4). This suggests that basil hairy roots extract significantly reduces hair loss and thus leads to denser hair.

CONCLUSION

Extracts from *Opuntia ficus-indica* and basil hairy roots are two cosmetic actives that are effective against skin irritation and hair loss, respectively.

Basil hairy root extract inhibits 5α reductase II

Since 5α reductase is the enzyme which converts testosterone to dihydrotestosterone, the hormone responsible for hair miniaturization in androgenic alopecia (Figure 3A), the ability of basil hairy roots extract to inhibit the activity of 5α reductase II, the enzyme isoform present in scalp hair follicles, was investigated. In this *in vitro* study, cell extracts containing 5α reductase II from transfected human embryonic kidney cells were used. The inhibitory potency of basil hairy roots extract on 5α reductase II enzymatic activity was measured. As a positive control Finasteride, an approved 5α reductase inhibitor and synthetic drug to treat baldness in men, was used. The results for treatment with basil hairy roots extract showed a clear concentration-dependent inhibition of 5α reductase II with an IC₅₀ value of 2.62 mg/ml (Figure 3B). This indicates that basil hairy roots extract might be able to reduce hair loss in people with androgenic alopecia.

Basil hairy root extract reduces hair loss

In a clinical study conducted for two months on a mixed panel with mild to moderate hair loss, basil hairy roots extract displayed the capacity to significantly reduce hair loss. Volunteers applied a fluid containing 1% of the basil hairy roots extract every evening over a period of 2 months. Results showed that the numbers of daily lost hair dropped by 26% after the first month and 31% following the second

Therefore, they are excellent ingredients to specifically target men's skin and hair care concerns. With the male grooming sector steadily growing, one can expect more tailored cosmetic actives for men's care in the future.

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