KEY POINTS

- Oxidative stress caused by UV radiation and pollution causes damage to the hair follicle and contributes to premature hair loss.
- In response, a yerba santa extract was assessed for its capability to protect hair follicles from oxidative damage and to reduce premature hair loss.

Hair Loss, Uprooted

Follicle Protection with Yerba Santa

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With the body, skin and scalp being the outermost boundaries of the body, they are exposed to a myriad of external stresses and pollution every day. Extensive studies over the years have shown that pollution and UV radiation can lead to premature skin aging, as they are some of the primary causes of reactive oxygen species (ROS).[1] These highly reactive molecules cause damage to DNA, such as the formation of 8-hydroxydeoxyguanosin (8-OHdG) or the oxidation of fatty acids or amino acids in proteins, thereby potentially causing DNA mutations and impairing cell membrane or enzyme function.

Detoxification of ROS is mediated by several cellular enzymes including catalase, superoxide dismutase or glutathione peroxidase.[2] A key transcription factor involved in

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the protection of cells from oxidative damage is nuclear factor erythroid 2-related factor 2 (Nrf2). This regulates a variety of genes such as detoxifying enzymes through an antioxidant response element in its promoter region.[2] The activation of Nrf2 as a protective factor was studied and led to the development of various products to protect the skin from stresses causing oxidative damage. These ROS detoxification systems are also present in scalp hair follicles, according to Haslam.

Despite the fact that ROS production by the mitochondria is essential for the physiological activities of the hair follicle, including hair growth,[3] it has been shown that oxidative stress promotes premature catagen development ex vivo, which can be prevented by Nrf2 activation.[4] Along this line, UV radiation induces hair follicle damage that leads to hair growth inhibition ex vivo.[5, 6] As a consequence, the cytotoxicity and hair growth inhibition induced by oxidative stress has been implicated in several hair loss disorders.[3]

Recent evidence presented at the 28th Congress of the European Academy of Dermatology and Venereology (EADV) showed that common air pollution comprised of particulate matter (PM) and heavy metals induce oxidative stress and damage cells in hair follicles, thereby causing premature hair loss.[7] These findings substantiate the need for the development of products that effectively and specifically protect hair follicles from oxidative damage caused by environmental pollutants and UV radiation.

In this study, an extract prepared from *Eriodictyon californicum* (yerba santa) was analyzed and studied in clinical trials for its protective effect on microdissected organ-cultured hair follicles. Being rich in antioxidative bioflavonoids, the extract was tested for its potential to protect hair follicles against oxidative damage caused by UVB radiation and pollutants, and thereby prevent the prolongation of the anagen hair growth phase.

Yerba Santa

Plants rich in polyphenolic compounds such as flavonoids have been shown to possess antioxidant activities[8] and present sources for the development of natural active ingredients for protection of the hair roots. *Eriodictyon californicum*, also known as yerba santa, mountain balm, bear's weed or gum bush, is an evergreen aromatic shrub that belongs to the plant family of Boraginaceae. Yerba santa typically grows in clonal stands, around one meter in height. Its oblong lanceolate leaves are dark green with a leathery texture and covered with shiny resin, while its flowers are white to light purple and bell-shaped.

Being native to California and Oregon, yerba santa has been regarded as a "holy herb" by the native population. In relation to this nickname, it has a long history of use in traditional medicine not only to treat asthma and respiratory tract infections, but also wounds, broken bones and sores.[9, 10]

Moreover, recent research has identified one of the major flavonoids of yerba santa, sterubin, as being neuroprotective against multiple toxicities of the aging brain.[11] Other polyphenolic constituents of yerba santa are the flavanones eriodictyol and homoeriodictyol, which possess antioxidant activities.[8] Thus, the efficacy of an extract prepared from the aerial parts of yerba santa to protect hair follicles from

Scalp massages with essential oils such as cedarwood, lavender, tulsi, thyme, peppermint and rosemary have been seen in several trails to help naturally promote hair growth.

Source: Medical News Today

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oxidative stress was tested in vitro and in vivo as described next.

Experimental Design

Protein carbonylation and oxidative stress: To assess the extract's protective effect against protein oxidation in vitro, human keratinocytes (HaCaT) were cultured in the presence of 0.5% yerba santa extract; the reference compound N-acetyl-L-cysteine (NAC, 2.5 mM); or left untreated for 24 hr. Oxidative stress was induced by adding 100 µM H_2O_2 for 30 min during continuous cell treatment with the test compounds. Cells were subsequently fixed with ethanol/acetic acid and carbonylated proteins were labeled with a specific fluorescent probe. The excess probe was removed by successive washes with phosphate buffered saline (PBS). Fluorescent signal intensity was recorded using a multiplate reader[a] and normalized to untreated cells.

C. elegans *and oxidative stress:* The protective effect of the extract on oxidative stress in a whole organism also was tested in the nematode *Caenorhabditis elegans* (*C. elegans*). The organisms were subjected to oxidative stress as described previously.[12] *C. elegans* were grown on agar plates containing nematode growth medium prepared with or without (control) 0.5 mg/mL yerba santa extract until larval stage 4 (L4). The organisms were subsequently washed with M9 buffer[13] and plated in 96-well plates at 60 per well in triplicates for each treatment.

C. elegans were left untreated or subjected to oxidative stress by the addition of 5 mM arsenite with dilution of 0.05 M $NaAsO_2$ in M9 buffer[b]. *C. elegans* locomotor activity was tracked using an infrared system and the movement score was calculated based on the number of times the light beam was disrupted by *C. elegans* in each well. Continuous measurements were performed for up to 60 hr.

Hair follicle protection upon UVB irradiation: To assess protective effects against oxidative stress and DNA damage, the extract was tested in hair follicles. Clinically healthy

[a] *BMG Labtech*

[b] *Honeywell International*

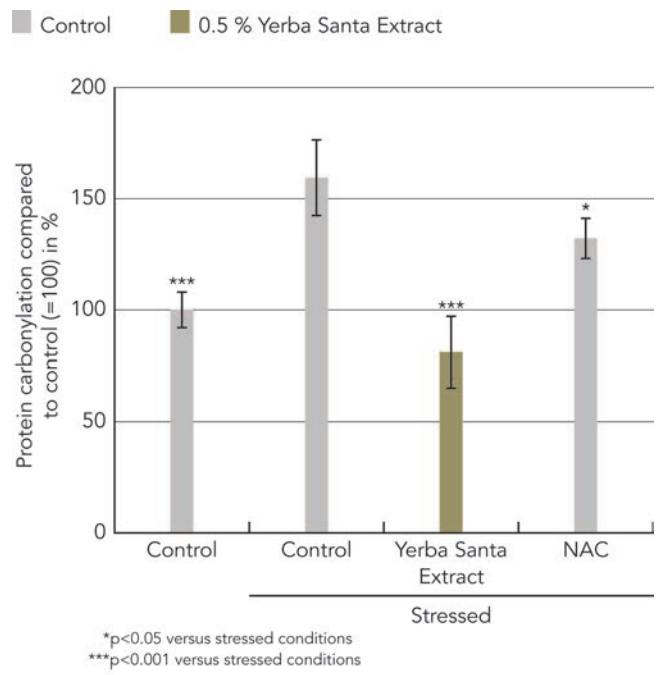

Figure 1. Experimental results of protein carbonylation and oxidative stress

human hair follicles (HF) were microdissected from follicular units obtained from the occipital scalp of a male donor undergoing hair transplantation and cultured, as previously described.[14] Anagen VI hair follicles used for further experiments were selected based on microscopic observation and the occurrence of growth within the first 24 hr of culture. Hair follicles were treated or not (control) with 0.1% yerba santa extract 24 hr after isolation, and treatment was renewed 72 hr and 120 hr after isolation.

Additionally, HF were irradiated with a low dose of UVB (20 mJ/cm^2) at 72 hr and 120 hr after isolation, as previously described.[5, 6] HF elongation was measured 24 hr, 72 hr, 120 hr and 148 hr after isolation. After 148 hr of culture and treatments at the indicated times, HF were collected and embedded for optical coherence tomography (OCT). Phosphorylation of Nrf2 and the formation of 8-OHdG served as indicators of oxidative stress and DNA damage, and were assessed by immunofluorescence staining of HF sections. Hair follicle scoring and

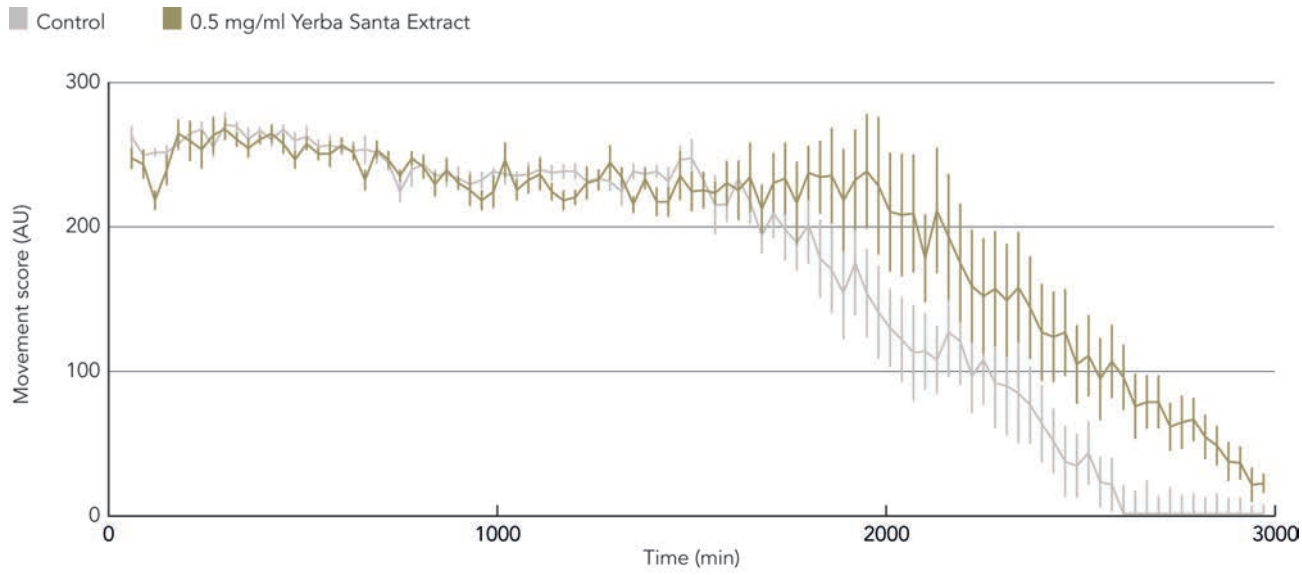

Figure 2. Experimental results of *C. elegans* and oxidative stress

Without functional intrinsic protection mechanisms and the right care, the hair follicle can be easily damaged.

determination of the HF stage was performed as described previously.[14]

Hair loss and regeneration in vivo: Clinically, the effect of yerba santa extract on hair loss and regeneration was assessed in a double-blind, randomized, placebo-controlled clinical study. For this, 56 healthy male and female volunteers (aged 18-68 years) with hair loss of variable causes were included in the study. Following a wash-out phase of 48 hr before the initial study visit, during which the volunteers did not wash their hair, hair density was evaluated using a phototrichogram microcamera[c].

Moreover, a cosmetic trichogram was carried out. For this, an average of 25 hairs including the roots was plucked from each volunteer for microscopic analysis and the determination of the number of anagen and telogen hair. The subjects applied a hair serum containing 2% yerba santa extract or a placebo product once daily for 150 days. A second wash-out phase of 48 hr was carried out prior to the final study visit, followed by the determination of the test parameters and plucking of hairs.

Experimental Results

Protein carbonylation and oxidative stress: As noted, specific staining of carbonylated

[c] *TrichoScan, Tricholog GmbH*

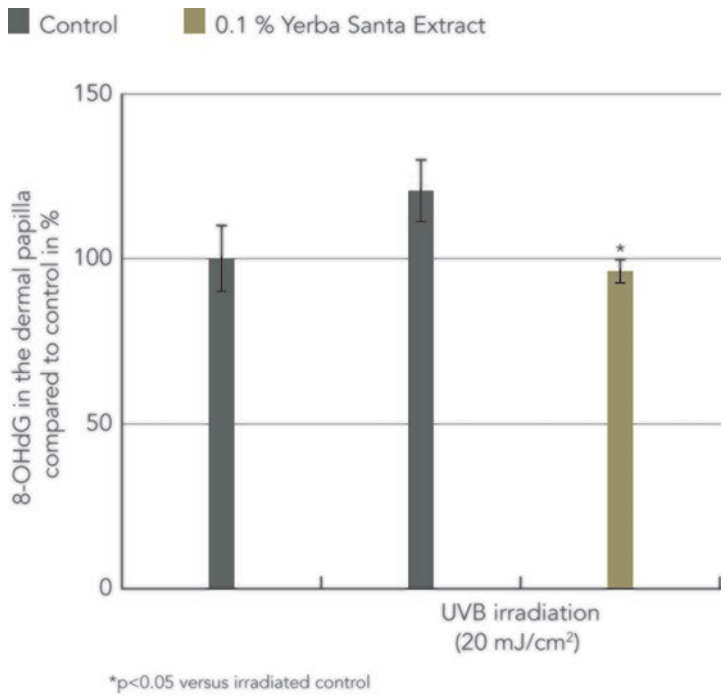

Figure 3. Experimental results of 8-OHdG

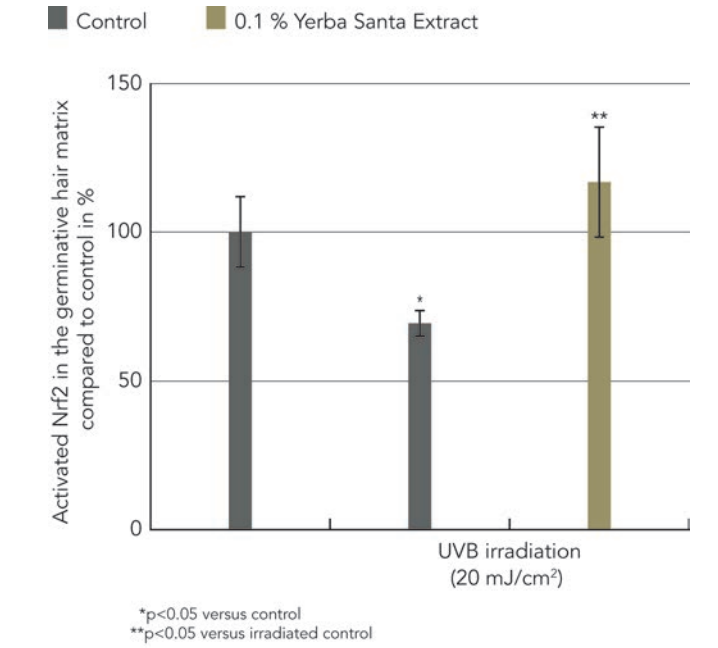

Figure 4. Experimental results of Nrf2

proteins was performed following 24 hr of pretreatment with the test compounds or control prior to 30 min of oxidative stress. Yerba santa extract tested at 0.5% had a significant protective effect against protein oxidation, leading to a 78% reduction in carbonylation, compared with stressed cells (see **Figure 1**). This effect was stronger than the effect of the reference compound NAC, which was also protective but to a lower extent of 27%.

C. elegans *and oxidative stress:* As described, *C. elegans* that had been grown on media containing yerba santa extract were subjected to oxidative stress by arsenite treatment.[12] The movement score was determined for up to 60 hr during the oxidative stress induction, being reflective of the entities' locomotor activity and viability. Exposure to 0.5 mg/mL yerba santa extract during maturation of the nematodes led to a prolongation of their activity upon arsenite stress (see **Figure 2**). This result exemplifies that the media containing the extract offered protection against the negative effects of oxidative stress caused by arsenite treatment, compared with the control.

Hair follicle protection upon UVB irradiation: In the case of UVB-irradiated hair follicles, the radiation caused oxidative stress that was reflected by a marked increase in oxidative DNA damage, designated by the formation of 8-OHdG and a decrease in Nrf2—a transcription factor facilitating responses to oxidative stress and cellular detoxification. Pre-treatment of HF with yerba santa extract led to a significant reduction in 8-OHdG in the dermal papilla (see **Figure 3**).

Moreover, the activation of Nrf2 was increased in the germinative hair matrix following pre-treatment with yerba santa extract before UVB irradiation (see **Figure 4**). This was also observed in the per-cortical hair matrix and outer root sheath (data not shown), and leads to an increased ability of the HF cells to respond to oxidative stress.

Determination of the hair cycle stage confirmed the protection of hair follicles from premature entry into the catagen phase caused by UVB irradiation (see

Figure 5). Taken together, these results indicate the extract protected hair follicle cells against environmental stress.

Hair loss and regeneration in vivo: In the clinical study, from the average 25 plucked hairs analyzed, the number of hairs in the anagen phase increased significantly by 31.5%—i.e., from an average of 16.2 hairs at the beginning of the treatment, to an average of 21.3 hairs following 150 days of treatment with yerba santa extract. In comparison, the placebo treatment increased anagen hairs from an average of 17.2 to just 18.1 after 150 days (see **Figure 6**).

Furthermore, treatment with yerba santa extract for 150 days led to a significant increase in the ratio of anagen versus telogen hairs, which is indicative of increased hair growth (see **Figure 7**). In line with this, the overall number of hairs increased by 13.2% at the end of the treatment, a significant improvement compared with the initial condition (data not shown).

Microscopic analysis of plucked hairs at the beginning and end of the treatment with yerba santa extract further showed a thickening of the hair root (see **Figure 8**). Overall, the results of this study showed that yerba santa extract strengthened the hair root, shifted hairs to the anagen growth phase and increased hair density.

Conclusion

The hair is exposed to a variety of potentially damaging factors every day. These include those that are intrinsic, such as genetics, metabolism or hormonal imbalances; but also, to a great extent, extrinsic—i.e., chemicals, pollution and UV radiation. Particulate matter, which comprises common air pollution, was recently shown to induce oxidative stress and to cause cell damage in hair follicles, ultimately leading to hair loss.[7] Similarly, UV irradiation has been shown to cause the formation of ROS, which damages the hair follicle.[15]

Without functional intrinsic protection mechanisms and the right care, the hair follicle can thus be easily damaged. Damage to the hair root, in turn, can

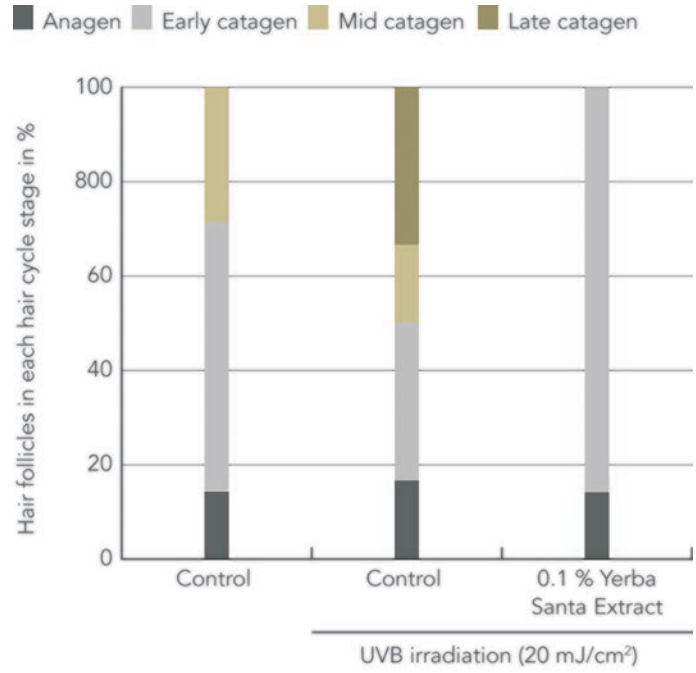

Figure 5. Experimental results of the hair follicle and UVB irradiation

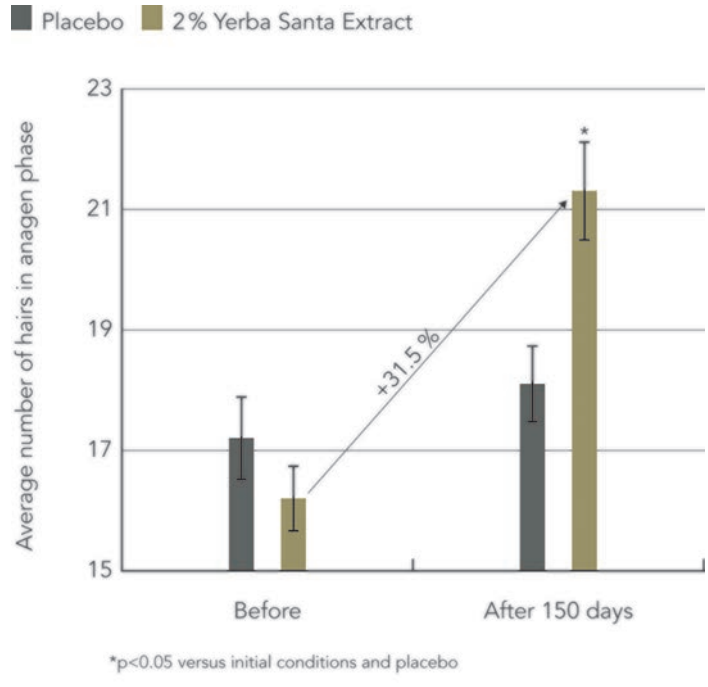

Figure 6. Placebo treatment for anagen hairs

cause premature hair loss due to the induction of the catagen hair growth phase—the regression phase during which the shaft is no longer produced. It is therefore crucial to support the hair at the root by offering protection from such stresses and subsequent oxidative damage.

In this work, the protective effect of an extract prepared from yerba santa on hair follicle cells were assessed based on its high content of polyphenols and flavonoids, such as the bitter-masking flavanones eriodictyol, homoeriodictyol and sterubin.[16, 17] Flavonoids are known to possess a variety of properties including antioxidant and anti-inflammatory activities.[8] In particular, homoeriodictyol has been shown to protect cells from oxidative stress by activating the major antioxidative pathway involving the transcription factor Nrf2.[18]

Moreover, there is evidence that eriodictyol possesses radical-scavenging properties[19] and further protects keratinocytes from UV-induced apoptosis.[20] The experiments performed in this study confirmed the antioxidant and UV-protective properties of the yerba santa extract. It effectively prevented protein carbonylation caused by the oxidizing agent H_2O_2, reducing oxidative damage to human keratinocytes. The previously described negative effect of pollutants, such as arsenite, was also ameliorated by yerba santa, confirming the protective effect observed in the cellular model. In line with this, oxidative damage such as the formation of 8-OHdG caused by UVB irradiation was prevented in human hair follicles upon treatment with the extract, while Nrf2 was activated.

These findings further translated to a positive effect on hair growth in vivo. In healthy volunteers presented with hair loss, daily treatment with a hair serum containing yerba santa extract led to a significant shift to the anagen growth phase and an increase in hair root thickness. The resulting increase in hair density further suggests effective protection of hair follicles in the human scalp from daily external stresses.

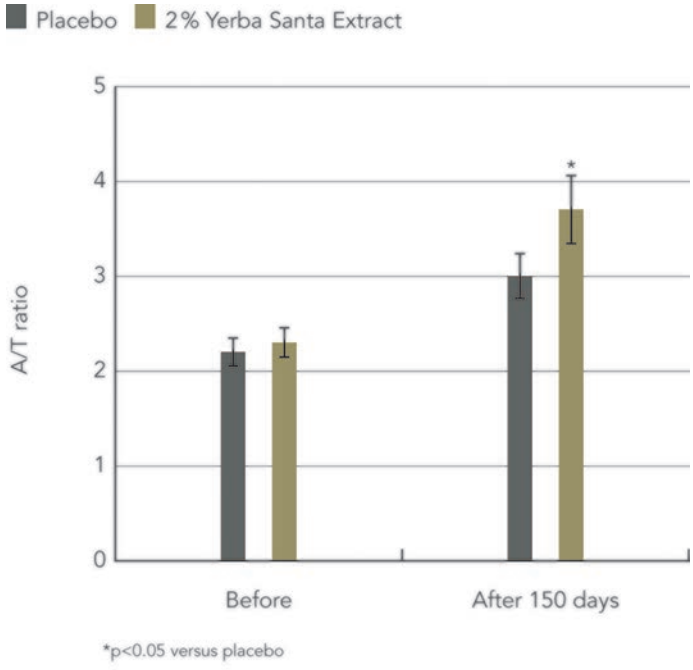

Figure 7. Experimental result of anagen versus telogen hairs

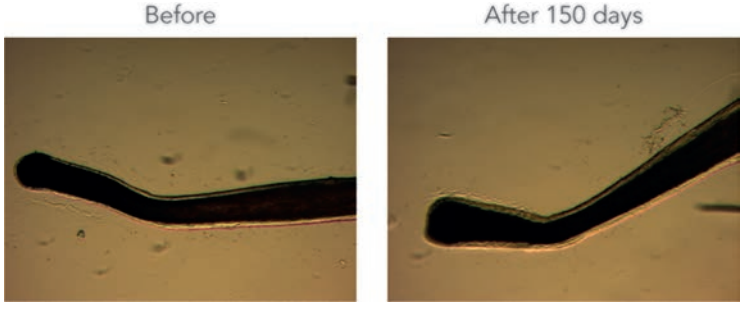

Figure 8. Microscopic analysis of plucked hairs treated with yerba santa

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