Ecocert Extract from Opuntia Cactus with Proven Activities

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Abstract

The pads of the cactus Opuntia are known as nopales and are used in Mexico as a dietary supplement to promote weight loss and as a medicine to treat inflammation and pain. Purified components of the Opuntia cactus were used for the development of a cosmetic ingredient that provides long-lasting hydration to skin even in rinse-off applications. This ingredient also has remarkable soothing properties which could be shown in a fascinating cell culture system with nerve cells. The Opuntia cactus ingredient was found to inhibit the depolarisation of sensory nerve cells when under stress. The active's protection against UV was shown in vivo. The synergistic activities can be applied in sun care products and skin care products to soothe sensitive and dry skin. Opuntia is sold commercially as AquaCacteen nc by our company.

Introduction

The most important factors for sensitive skin are skin dryness and atopic skin, meaning skin that is hyper-reactive towards allergens. This article describes a preparation of the Opuntia cactus that helps against sensitive skin in two different ways: it increases skin hydration and it specifically reduces the itch sensation and local inflammation. The latter phenomena are the consequence of a 'crosstalk' between the stratum corneum, free sensory afferent nerve endings and mast cells (Figure 1, next page). A stimulus such as a pruritogenic substance or inflammatory mediators or cytokines produced by keratinocytes act either directly on a subset of specialised nerve endings, called C-fibres, inducing an action potential or on mast cells inducing degranulation. The action potential leads to the evocation of nociceptive reflexes and pain behaviour and to the release of neuropeptides such as substance P and the calcitonin gene-related peptide (CGRP). These neuropeptides induce dilation of the surrounding capillaries and local inflammation and also degranulation of mast cells. Degranulation liberates histamine, which, in turn, initiates itch and local inflammation by stimulating the C-fibre nerve endings.

To the genus Opuntia belong about 200 – 300 cactus species 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 term 'nopales' is used for the flattened stem segments that are morphologically incorrectly designated as cactus leaves. The dried powdered cactus leaves are used as a nutritional supplement. They are a rich source of minerals, pectins and flavonoids[1]. There are several scientific publications that report the analgesic action and anti-inflammatory properties of nopal extract[2].

Materials and Methods

Preparation of the Cactus Extract 'AquaCacteen nc'

Leaves of the species Opuntia Ficus indica, organically grown and certified, which had been carefully dried at relatively low temperatures, served as a source material. The material was taken up in a phosphate buffer solution and digested with a special enzyme mixture. The final product was obtained after passage through different filter devices and cross flow filtration through a 10 kDa membrane. For standardisation of the extract the lead substance piscidic acid (Figure 2, next page) was analysed by HPLC-MS. Piscidic acid is a chelator of free iron ions.

Protection Against UVA Irradiation

Human keratinocytes (HaCaT) were irradiated with UVA for 25 minutes at a dose of 1125 kJ/cm^2 ± 'AquaCacteen'. Cell growth was measured 24 and 48 hours later with the MTT assay.

Coculture Model with Sensory Neurons and Keratinocytes

Rat sensory neurons were cultivated in a plate with 96 wells in a coculture medium. After 10 days, normal human keratinocytes were seeded in each well. After 2 days of coculture, supernatants were changed by coculture medium alone or with the test compound and cells were incubated

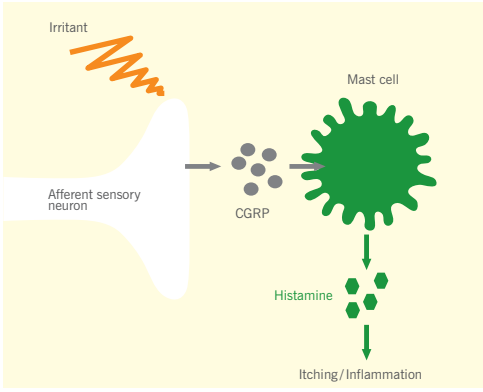

Figure 1. Sensory system of the skin

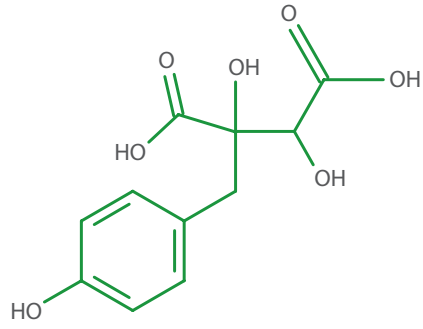

Figure 2. Lead substance piscidic acid that was used for standardisation of the extract.

for 30 minutes. At the end of this incubation, the cells were stimulated for 20 minutes with 10^{-6} M capsaicin. Then the supernatants were recovered and frozen to analyse CGRP by ELISA. Lidocaine at 10^{-6} M was used as positive control.

Study on Skin Hydration

Shower gels with different concentrations of 'AquaCacteen nc' were tested in a study over 1 week with 20 women from the ages of 23 to 49. The products were applied once daily on separate areas on the inner side of the forearm. Hydration was measured with the Corneometer CM 825 PC.

Results and Discussion

The nopal preparation 'AquaCacteen nc' was first studied in vitro with keratinocytes. The human cell line HaCaT was used to verify if nopal contains antioxidants that confer a protective effect against irradiation with UVA. The MTT-assay was used to measure the concentration of metabolically active cells[3]. The applied irradiation dose and irradiation time normally leads to a cell death of about 50%. In this experiment the survival rate in the normal cell medium was 42% after 1 day and 25% after 2 days (Figure 3). Keratinocytes in the medium with 1% 'AquaCacteen nc' clearly showed a higher resistance against UVA treatment and after 2 days regeneration. Survival after 1 day was at 53% and then the culture started to grow again reaching 61% of the amount of metabolically active cells that were present before irradiation. Thus in Opuntia cactus ingredients are present that help keratinocytes to better tolerate UVA. Also the Opuntia lead substance, piscidic acid, could contribute to this protective effect. Piscidic acid neutralises free iron ions that otherwise would initiate the formation of hydroxyl radicals through the Fenton reaction. Ferritin, the body's own iron chelator, is decomposed upon UV-radiation.

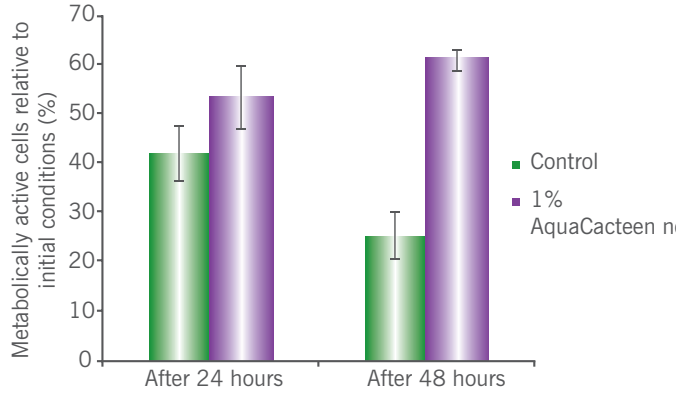

Figure 3. Protection of keratinocytes after UVA irradiation. Human keratinocytes (HaCaT) were irradiated with a LD50 dose of UVA. After 1 and after 2 days the amount of metabolically active cells was measured by the MTT assay.

A cell culture test system with sensory nerve cells and keratinocytes was used to demonstrate a soothing, anti-inflammatory effect. C-fibre nerve cells were cultured together with keratinocytes. Capsaicin, the pungent active in chilli pepper, is a strong skin irritant and was therefore used in this test system as a stimulus to induce an action potential. Stimulation of the C-fibre nerve endings was recorded by measuring the concentration of the released neuropeptide calcitonin gene-related peptide (CGRP). Reduced CGRP liberation in the presence of capsaicin indicates a soothing activity. Lidocaine, a local anaesthetic that blocks the signal at the endings of sensory nerves, was used as a positive control. Stimulation of the coculture with capsaicin resulted in a prominent release of the neuropeptide CGRP (Figure 4). The

positive control lidocaine reduced CGRP release by 61% and a solution of 0.3% nopal preparation by 42%.

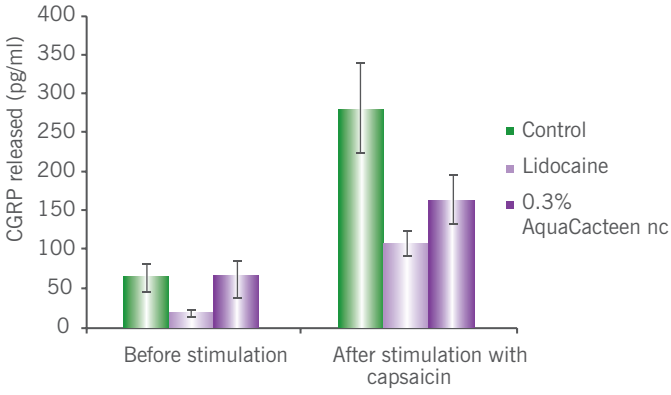

Figure 4. Soothing effect of AquaCacteen nc in a coculture model with sensory nerve cells and keratinocytes. The concentration of the neuropeptide CGRP in the culture supernatant was measured before and after stimulation with capsaicin. The analgesic lidocaine was used as positive control.

Dry skin favours the development of sensitive skin reactions. The nopal preparation was therefore tested for beneficial effects on skin hydration. To show a moisturising effect even in a rinse-off product, different concentrations of 'AquaCacteen nc' were tested in shower gels. A distinct dose-dependent increase in skin hydration was obtained (Figure 5). The product with 2% 'AquaCacteen nc' increased hydration by 16% after 1 week of application.

Conclusion

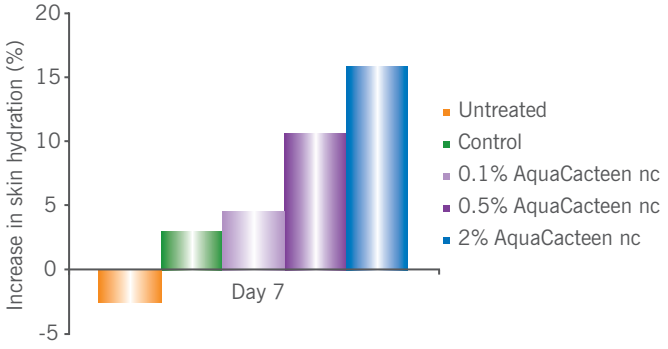

Figure 5. Improvement of skin hydration. Shower gels with different concentrations of AquaCacteen nc were used during 7 days.

Members of the cactaceae family are biologically adapted to resist strong sunlight, extreme drought and big differences in day / night temperatures. The commercially available Opuntia cactus was used as source material to exploit this extraordinary protective activity. The final product 'AquaCacteen nc' was found to protect skin cells against UV light. Skin hydration could be increased with 'AquaCacteen nc' and a soothing effect could be demonstrated in a cell culture model for sensitive skin.

References

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2. Park E.-H., Kahng J.-H., Paek E.-A. (1998) Studies on the pharmacological actions of cactus: Identification of its anti-inflammatory effect. Arch. Pharm. Res. 21: 30 – 34

3. Mosmann T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65: 55 – 63

Authors' Biography

Dr. Daniel Schmid received his Ph.D. in biochemistry from the University of Zürich, Switzerland. He worked for several years in the Research Center of Nestlé at Vers-chez-les-Blanc, where he studied health effects of probiotic bacteria. He is presently responsible for research in Mibelle Biochemistry, a business unit of Mibelle AG Cosmetics. Mibelle Biochemistry develops and produces active ingredients for skin care products.

Dr. Frank Gafner is a pharmacist and received his Ph.D. in phytochemistry at the University of Lausanne with Prof. Hostettmann. After a post doc at the University of California (Irvine) in the field of sensitising natural products, he joined the environmental and trace analysis group of Ciba-Geigy in Basel. After 15 years in R&D at Pentapharm, Alpaflor and Induchem, he recently joined Mibelle Biochemistry, where he applies his experience with natural products in the product development.

Dr. Fred Zülli is a molecular biologist and undertook his Ph.D. at the ETH Zürich on genetic engineering of thermostable enzymes. He spent his post doc at the Nestec Research Center on molecular biology and genetics of commercial baker's yeast strains. Since 1991, Dr. Fred Zülli has been head of the business unit Mibelle Biochemistry, responsible for development, production and marketing.