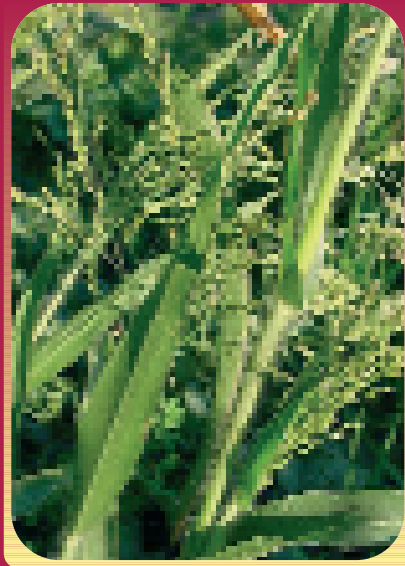




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**Millet Oil for Skin and Hair Care**





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## Millet Oil for Skin and Hair Care

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### Introduction

**M**illet is a general term for a wide range of small-seeded annual grasses that have been grown for thousands of years on all continents. Even today, millets are still the main source of food in some countries in Asia and Africa. Millet seeds are relatively rich in oil and contain essential fatty acids and exogenous essential amino

acids as well as different mineral substances.

Throughout the ages, millet has always been associated with very favourable properties. For example, it promotes a healthy skin, vitalizes hair growth, improves hair gloss, and strengthens fingernails. The millet oil contained in the millet seeds surely has a very positive influence on all this.

#### ■ Production and efficacy of millet oil

E. Zwicky AG in Müllheim-Wigoltingen Switzerland produces millet oil from genetically unmodified proso millet (*Panicum miliaceum L.*) which is grown in the western states of the USA (North and South Dakota, Minnesota, Colorado, Nebraska, Kansas and Arkansas) partly in biological farming. The millet seed is first subjected to a mild, non-oxidative drying process known as collatization (from the German: collatieren®), which results in a stabilized whole grain product (Goldhirse®, literally golden millet). Extraction of the collatized millet with ethanol then yields extremely pure millet oil.

This millet oil contains 64.9% linoleic acid and 1.4%  $\alpha$ -linolenic acid as well as tocopherol (vitamin E) and vitamin B<sub>6</sub>. Tests carried out with this oil on human skin explants (*in vitro* skin models) showed good skin compatibility and a protective function against possible skin damage through skin irritants such as croton oil. Regeneration of the epidermis after previous skin damage was also promoted. Efficacy studies following oral intake of encapsulated millet oil (HIRSANA® food supplement, gold millet oil capsules) showed a significant improvement of hair gloss as well as an increase in hair thickness after three months following an intake twice a day of 200 mg millet oil.



Fig. 1-3 Proso millet (*Panicum miliaceum*), Foxtail millet (*Setaria italica*), Sorghum millet (*Sorghum bicolor*) (Source Fig. 2+3: Detlev Franz).

The studies furthermore confirmed the complete compatibility of the millet oil capsules.

#### ■ The variety of millet types

Millet was one of the first cereal crops cultivated by mankind and is even today grown on all five continents. As typical summer cereal with short vegetative period, millet is also suitable for growing in farming areas with very dry and warm climates.

In the middle ages, millet mash was the staple diet of poor people. Millet was largely replaced at the end of the 18<sup>th</sup> century following the introduction of the potato. Because of its short growing time, millet is often sown on wheat and rye fields already harvested in order to supplement wheat and rye stocks.

Millet is the general term for a series of small seeded cereals. The three most important types are proso, foxtail and sorghum millet (Figs. 1-3). The name foxtail describes the appearance of the seed head (panicle). These types of millet originate from middle Asia. Foxtail millet spread to China, where it has been grown for 6000 years, while proso and also foxtail millet found their way to Europe and were grown there even before rye and wheat. Prehistoric excavations in France as well as findings in pile dwellings in Italy have brought to light 3000-year-old residues of foxtail millet.

Sorghum millet (*Sorghum bicolor*) has been grown in Egypt for over 4000 years.

And from there originates the traditional belief that millet is poor man's food. The words »Millet is poor man's corn« can be found in ancient Egyptian manuscripts. Today, sorghum millet is used primarily as forage.

Restricted possibilities for cultivation, low crop yields and limited possibilities for use have all contributed to millet's poor reputation. Because proso millet (*Panicum miliaceum*) and foxtail millet (*Setaria italica*) like oats and rice do not contain gluten, these cereals have only limited suitability for baking purposes, but all the more for making blini, pancakes and dough cakes.

It is not surprising then that millet has nowadays practically disappeared as a source of human food in Europe. Foxtail millet is used for birdseed and sorghum millet in general as forage. On the other hand, proso and foxtail millet food products are ideal for people who suffer from coeliac disease (gluten intolerance).

#### ■ Rough husk – valuable kernel

The seed heads of millet can grow up to 60 cm in length. Millet seeds are relatively small and have a diameter of about 1.5 mm and a weigh about 5 mg. Carbohydrates, 70%, are the main constituents of millet seeds. Besides this, the seeds contain about 10% protein, 3 to 5% oil and 1.6% mineral substances (phosphorus, fluoride, iron, calcium and silicon) as well as tocopherol and vitamin B<sub>6</sub>. The water content is about 12%. The

favourable effect that millet has on skin, hair, and nails surely has to do with the content of essential fatty acids. In addition, millet contains all the exogenous essential amino acids.

As a cereal, millet is relatively rich in oils (2 to 4%). Only oats have an oil content that is higher (about 7%). Very important for millet is the exceptionally high content of linoleic acid, which makes up two thirds of the total oil content. Linoleic acid is an essential fatty acid that has to be obtained from the diet because the body cannot produce its own supplies. Besides linoleic acid, other essential fatty acids such as  $\alpha$ -linolenic acid, eicosapentenoic acid, docosapentenoic acid and docosahexanoic acid are also present (Table 1).

#### ■ Millet oil for cosmetic applications

For the *in vitro* tests and for the studies involving oral intake by test persons, millet oil was used that had been produced from Goldhirse® by ethanolic extraction. For this purpose, selected, genetically unmodified proso millet of the *Panicum miliaceum* L. variety was used. This is grown in North and South Dakota, Minnesota, Colorado, Nebraska, Kansas and Arkansas, partly in biological farming. The warm summer, semiarid continental climate favours millet cultivation and promotes the quality of the millet seed. Harvesting is possible as early as 60 to 80 days after sowing. The millet obtained as threshed seed has an oil content of 2 to

4%, depending on the growing season. The process used to treat the seed prior to oil extraction has a decisive influence on the quality of the millet oil obtained. The especially mild collatization process (collatieren®) developed by *W. Kollath* allows debittered Goldhirse®, ground millet, millet flakes or millet flour to be stabilized as whole grain products for a long period of time (1). This process is employed exclusively by *E. Zwicky AG, Müllheim-Wigoltingen, Switzerland*, and is the source of the millet oil used for these studies. **Fig. 4** shows the appearance of the different products after each individual processing step.

**Table 1** summarizes the results obtained from the analytical determination of the main constituents, fatty acid composition, and vitamin content of the millet oil. The results confirm the high content of 64.9% for linoleic acid and 1.4% for  $\alpha$ -linolenic acid.

Linoleic acid plays a very important role in regulating the skin's moisture content. The gaps between the individual cells (intercellular spaces) of the horny cell layer and the upper granular layer are filled with lipids. The lipids behave as a kind of sealing substance and enable the horny cell layer to function as a protective permeability barrier.

This skin barrier minimizes the so-called transepidermal loss of water (TEWL), and thus stops the skin from drying out. It also prevents the penetration of harmful substances and microorganisms.

The epidermal lipids forming the barrier are composed of ceramides, cholesterol and free fatty acids, a major component of which is linoleic acid. The ceramides again consist mainly of long chain fatty acids derived above all from linoleic acid.

A deficiency of linoleic acid leads to a deficiency in the structural lipids in the epidermis that depend on linoleic acid. The increased loss of moisture causes the skin to become dry and then scaly with consequent loss of elasticity and stability.

If linoleic acid is applied to the skin in the form of a skin-care product or a cream, it can be directly incorporated into the cell membranes. The skin barrier is stabilized or a disrupted barrier function is normalized. The damaged skin undergoes regeneration and its functionality is renewed.

Besides moisture regulation, linoleic acid also has properties directly related to hormonal and immunological effects with accompanying anti-inflammatory functions. Finally, the prostaglandins PGE<sub>1</sub> and PGE<sub>2</sub>, complex fatty acid hormones that control pain and inflammatory behaviour, also originate from the linoleic acid family.

$\alpha$ -linoleic acid acts as a phospholipid building block and is an important structural component of all cell membranes. It increases their flexibility and is a precursor of the highly effective anti-thrombotic agent prostacyclin 1.

Clinical tests with millet oil carried out by *Nuzov et al.* (2) to treat suppurative wounds of patients suffering from dia-

Composition	Content		
	g/100 g	g/kg	mg/kg
<b>Main constituents:</b>			
Unrefined oil	95.6		
Unrefined protein	0.1		
Dietary fibre	0.1		
Water	1.5		
Peroxide number			< 10.0
<b>Fatty acids:</b>			
Palmitic acid		61.8	
Palmitoleic acid		1.2	
Heptadecanoic acid		< 0.5	
Heptadecene acid		< 0.5	
Stearic acid		20.8	
Oleic acid		183.3	
Linoleic acid		649.0	
$\alpha$ -Linolenic acid		14.0	
Parinaric acid		< 0.5	
Arachic acid		9.5	
Eicoseneic acid		4.5	
Eicosapentic acid		0.7	
Behenic acid		3.5	
Erucic / Docosic acid		1.1	
Docosapeic acid		2.1	
Docosahexane acid		2.6	
Saturated fatty acids, total		95.6	
Unsaturated fatty acids, total		858.1	
- Monounsaturated fatty acids, total		189.1	
- Polyunsaturated fatty acids, total		669.4	
<b>Vitamins:</b>			
Tocopherols, total			1663
- $\alpha$ -Tocopherol			190
- $\beta$ -Tocopherol			< 0.5
- $\gamma$ -Tocopherol			1120
- $\delta$ -Tocopherol			353
B <sub>6</sub>			0.16
<b>Minerals:</b>			
Phosphorus			1000
Fluoride			50
Iron			20
Calcium			20

**Table 1** Composition of millet oil. (Source: E. Zwicky AG, Switzerland).

## MILLET OIL

betes mellitus have demonstrated both anti-inflammatory and skin regenerative effects.

#### ■ Dermatological tests on *in vitro* skin models

Human explants (*in vitro* skin models) were used to gain a deeper insight into the mode of action of millet oil following application to the skin. The use of these skin models for a dermatological investigation allows a detailed microscopic analysis of effects occurring in

deeper layers of the epidermis, in contrast to classical dermatological tests where only macroscopic assessment of the outer surface is possible.

The cells in the epidermis consist mainly of so-called keratinocytes. These undergo a continuous differentiation process, during the course of which the living cylindrical cells in the basal layer of the epidermis are gradually transformed into flat, horny cells without nuclei. The epidermis constantly renews itself through cell division (proliferation) in the lowest layer, the basal cell layer. In the course of the differentiation, the cells gradually

move towards the upper layers of the epidermis, from the prickle cell layer (also called spinous cell layer), to the granular cell layer, and then to the horny cell layer. At the same time the cells gradually die and are finally present as flat horny scales on the surface of the skin that are then ultimately shed.

The keratinocytes produce characteristic biochemical components depending on their differentiation status. The presence of particular structural proteins is specific for each individual phase of the differentiation. For example, the structural protein cytokeratin 14 is above all detected in keratinocytes in the basal to suprabasal layers, that is, in the very lowest layers of the epidermis. The presence of cytokeratin 14 is therefore characteristic for the lowest layers of the epidermis and can be used as marker for basal keratinocytes. Besides this, the protein is also associated with cell division. There are also several other structural proteins that are characteristic for other differentiation stages of keratinocytes. These structural proteins therefore also serve as markers for the further differentiation stages. For example, the two early differentiation markers cytokeratin 10 and involucrin can be detected in the cells of the prickle and the granular cell layers. In contrast, the late differentiation marker filaggrin is found mainly in the cells of the outermost granular to horny cell layers (Fig. 5).

#### ■ Tests for skin compatibility

For initial investigations, millet oil was applied to the *in vitro* skin models in dilutions ranging from 1 to 10% and left for a day. The skin models were then fixed and embedded in paraffin. Ultra-thin tissue sections were prepared and stained using different methods.

An initial histological staining was carried out using hematoxylin and eosin. The actual state of the skin can then be assessed using transmitted light microscopy. This staining method allows the identification of possible differences in morphology (skin structure) between untreated skin and skin treated with millet oil. The tests performed in this way however yielded no significant morpho-

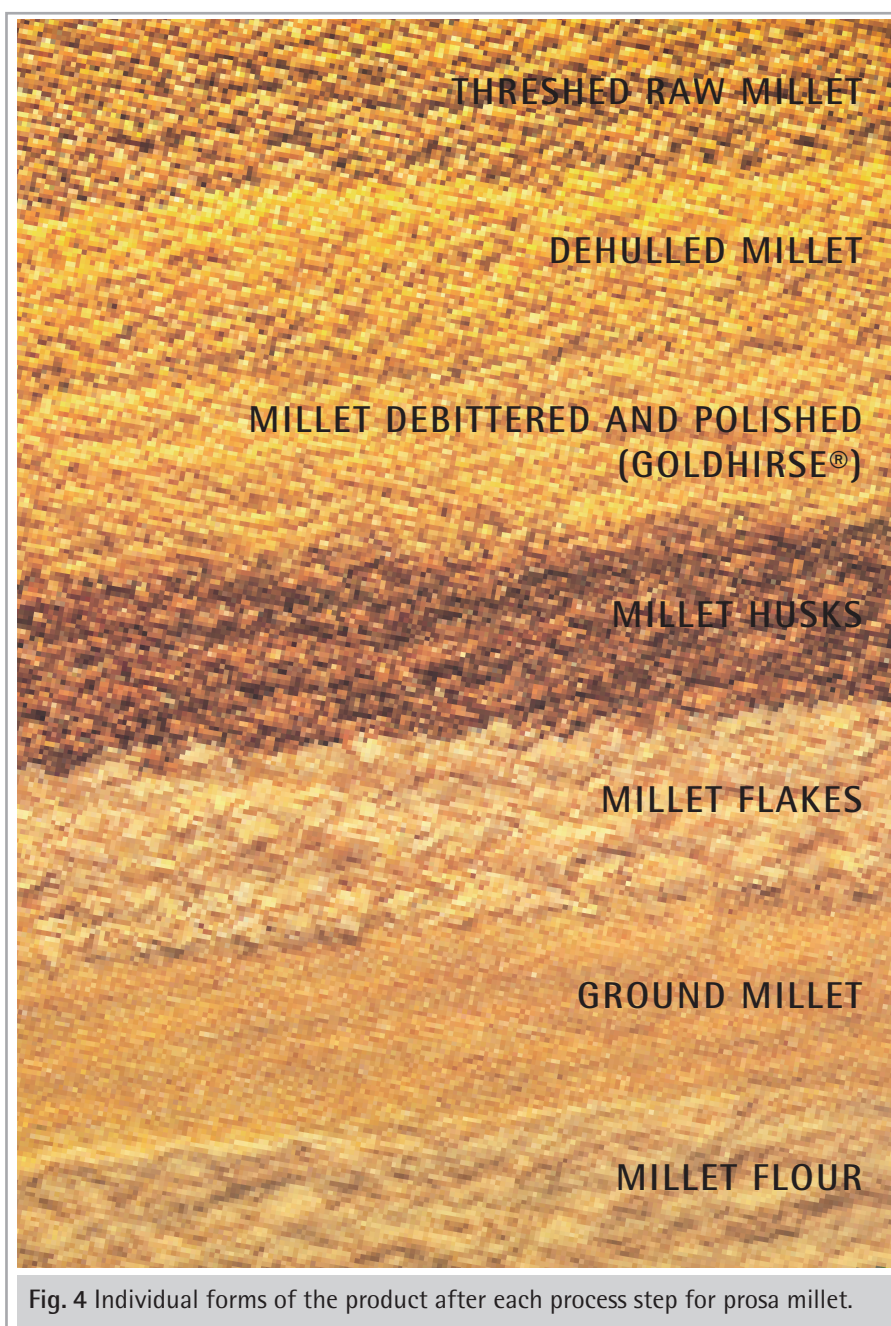


Fig. 4 Individual forms of the product after each process step for prosa millet.

logical differences between untreated and treated skin (Fig. 6). Clearly, millet oil, even at concentrations of up to 10%, did not cause any skin damage. This means that a high degree of skin compatibility can be assumed.

A second, immunohistochemical staining method was used in which certain biochemical components of the skin were specifically marked with the aid of appropriate antibodies and fluorescence dyes. This immunohistochemical staining method was used to detect the production of the structural protein cytokeratin 14 in the skin models treated with 1 to 10% millet oil. This second staining method also confirmed that the millet oil is very compatible with human skin. Furthermore, an increased production of the marker cytokeratin 14 was observed for the higher concentrations of millet oil (Fig. 7). Fluorescence signals for cytokeratin 14 were not only detected in the basal and suprabasal keratinocytes but also in the layers above. Since this protein is also associated with proliferation, increased cell division can be concluded as a result of the application of higher concentrations of millet oil.

■ Testing skin protective and regenerative properties

The initial studies had demonstrated that millet oil was very compatible with human skin and that it promoted cell division. Further experiments were then carried out to identify possible skin protective, anti-inflammatory and/or skin regenerative properties of millet oil. Two different experimental studies were performed to investigate this.

In the first study, *in vitro* skin models were once again pretreated for one day with different concentrations of millet oil (10%, 2.5% and 1%). Croton oil (2%), a powerful skin irritant, was then applied as a tissue-damaging agent. Afterwards, ultra-thin paraffin sections of the skin models were prepared. They were examined both by transmission microscopy (after histological staining with hematoxylin and eosin) and also by fluorescence microscopy (after immunohistochemical staining of the early differentiation marker cytokeratin 10) (Fig. 8).

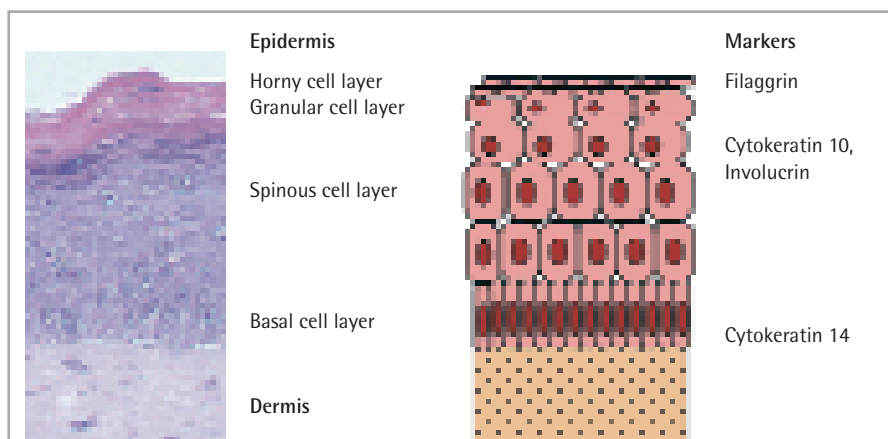


Fig. 5 Schematic diagram of the epidermis (outer layer of the skin) in comparison with a histological skin section. Individual differentiation markers are characteristic for the different layers of the epidermis.

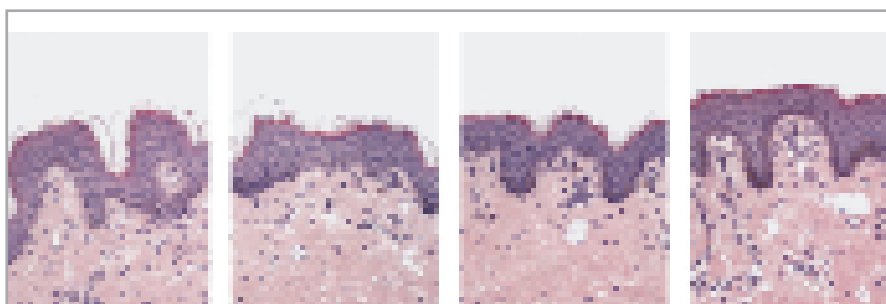


Fig. 6 Histological staining (hematoxylin and eosin) of *in vitro* skin models treated with different millet oil concentrations (from the left: untreated control, 10% millet oil, 2.5% millet oil, 1% millet oil). Cell nuclei are coloured blue to violet, and cytoplasmic constituents pink to red. A damaging effect of different millet oil concentrations on human skin is not observed.

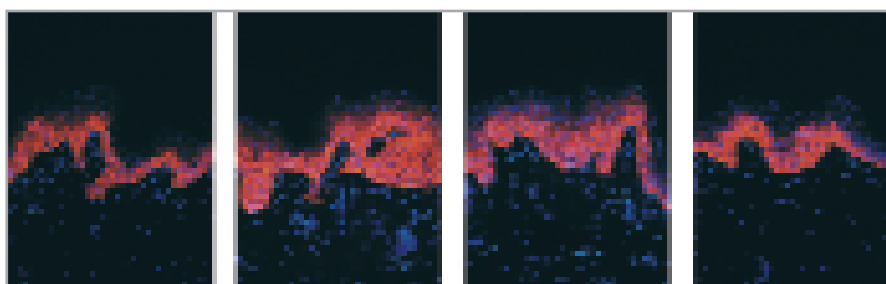


Fig. 7 Immunohistochemical staining of the marker cytokeratin 14 (which is associated with proliferation) in the *in vitro* skin models treated with different millet oil concentrations (from the left: untreated control sample, 10% millet oil, 2.5% millet oil, 1% millet oil). The cell nuclei of all cells fluoresce blue; the formation of the biochemical marker cytokeratin 14 in the cells can be seen as red fluorescence. A damaging effect arising from the different millet oil concentrations on human skin is not observed here either. At the same time an increased development of this marker is evident in the upper layers, which indicates increased cell division.

MILLET OIL

Both staining methods clearly demonstrated the positive effect that millet oil has on the human skin. When pretreated with higher concentrations of millet oil, the skin was largely protected against the damaging effects of the croton oil. This experiment thus proved that millet oil has valuable skin-protective properties. In the second study, the millet oil and croton oil were applied in reverse order. The *in vitro* skin models were first exposed to the aggressive, tissue-damaging croton oil (concentration 2%) for one day. The millet oil was then applied for one day in concentrations of 10%, 2.5% and 1%. The evaluation of the skin sections using hematoxylin and eosin staining and immunohistochemical staining of the skin component cytokeratin 10 also demonstrated the positive effects of millet oil. Skin regeneration from the damaging effects of croton oil was much better with higher concentrations of millet oil (Fig. 9). The results therefore clearly proved that millet oil has both skin-calming and skin-regenerative properties.

■ Compatibility tests and efficacy studies following oral intake

To check efficacy and compatibility, 200 mg of encapsulated millet oil enriched with vitamin E, vitamin B<sub>6</sub>, thiamine, riboflavin, pantothenic acid, biotin and zinc was administered to 39 test persons twice a day for three months. This millet oil is available on the market in capsules as the HIRSANA® gold millet oil food supplement. The test results confirmed excellent compatibility. Noticeable improvements were found in the external appearance of facial skin, fingernails and hair (4). Furthermore, the change in hair gloss that occurred from before the start through to the end of three-month period of intake of millet oil capsules was determined by measuring the gloss index of individual hair strands using gonio-photometric laser-light reflection according to the method of Wortmann et al. (5). At the end of the three-month intake period of these millet oil capsules, a significant increase in hair gloss was evident (Fig. 10). The hair gloss index improved significantly from 26.1% mea-

sured before the intake of the millet oil to 26.8% at the end of the three-month period (measured as the arithmetic mean with a standard deviation of 1.96\* with 95% confidence interval). The change in diameter of individual hairs of the test

persons was also measured before and at the end of the intake of millet oil capsules. Here again there was a slight increase in hair diameter at the end of the three-month intake period of millet oil capsules (6).

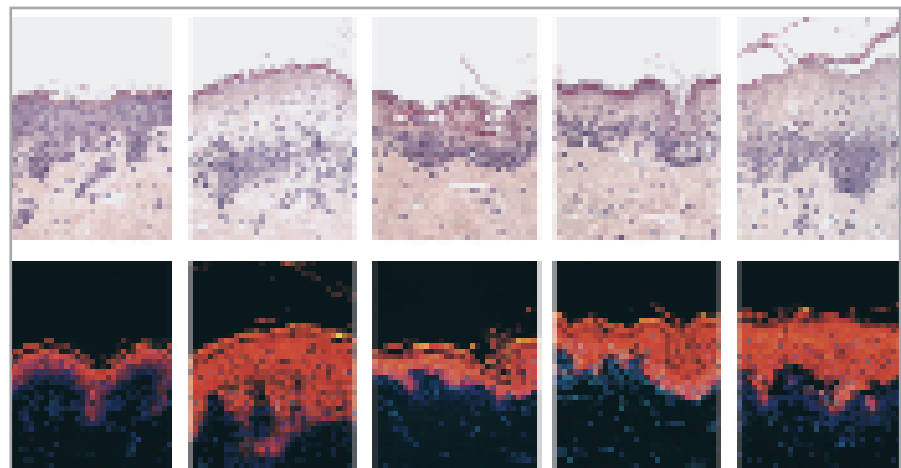


Fig. 8 Histological staining and immunohistochemical staining of the early differential marker cytokeratin 10 in the skin models treated. In order to detect the skin-protecting properties of millet oil, the skin models were first treated with millet oil before croton oil (a powerful skin irritant) was applied (from the left: untreated control sample, croton oil control sample, 10% millet oil, 2.5% millet oil, 1% millet oil). The higher millet oil concentrations effectively protect the skin against the tissue-damaging effect of the croton oil.

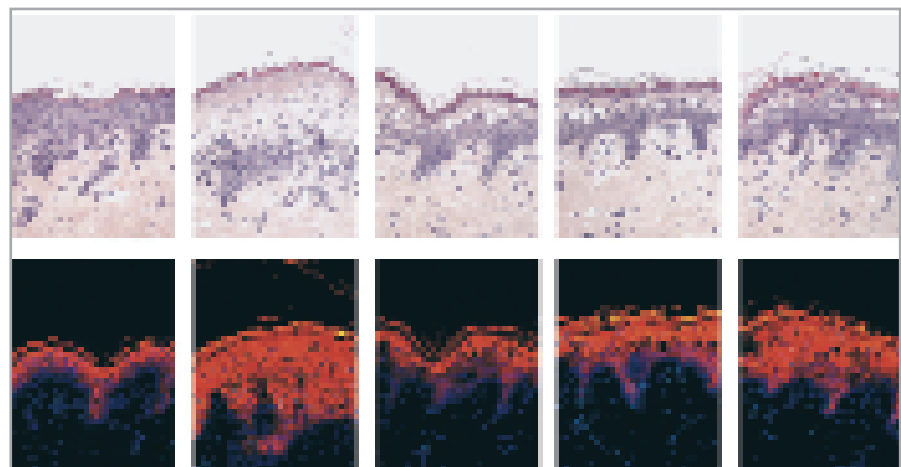
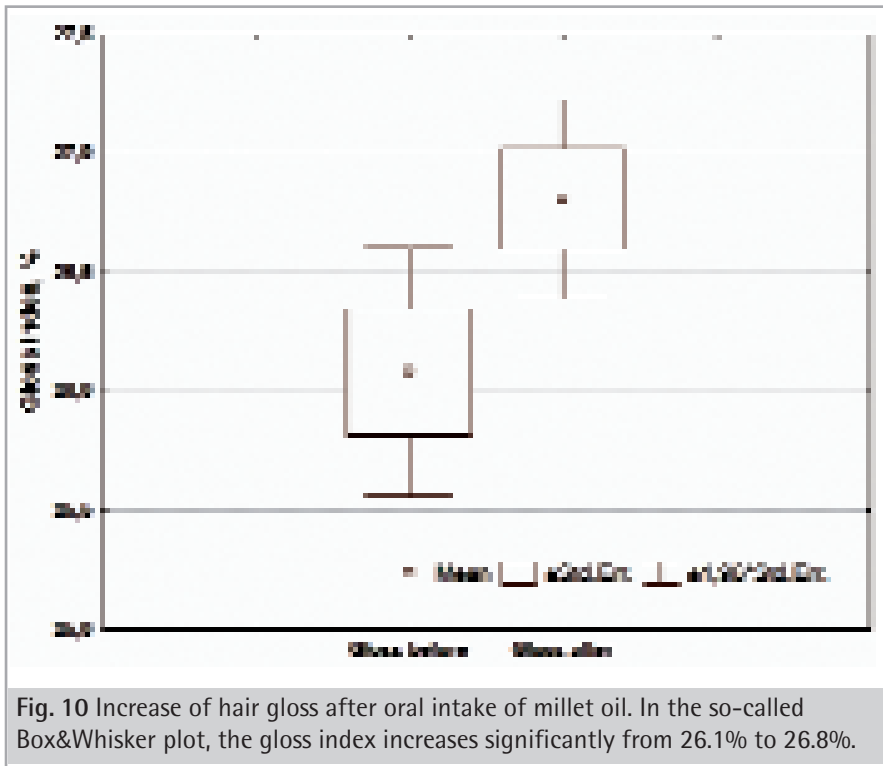


Fig. 9 Histological staining and immunohistochemical staining of the early differential marker cytokeratin 10 in the skin models treated. In order to detect the skin-calming and skin regenerative properties of the millet oil, the skin models were first damaged with croton oil before the different millet oil concentrations were applied (from the left: untreated control sample, croton oil control sample, 10% millet oil, 2.5% millet oil, 1% millet oil). After application of higher millet oil concentrations, the skin shows a good regeneration from the damaging effects caused by the croton oil.



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