

“yes” or “no” for questionnaire items before the test food intake, and evaluated the state of the skin in five grades (improved, slightly improved, no change, slightly worsened, worsened) after the end of the test food intake period.

Subjects enter the state of taking the test food, physical condition and other information every day in their diary.

The statistical analysis was a paired t-test used to assess intergroup differences of the changing rate of values determined before and at 1,2,3 and 4 week after the start of test food intake.

- **Evaluation of whitening effects**

The inhibitory and ameliorative effects of the test food on UV-induced pigmentation in the skin were evaluated by intergroup comparison of the values before and at 1, 2, 3 and 4 week after the start of the test food intake, as well as the data of questionnaires before and after the test food intake period, between the test food groups and control group.

The measures with the spectro colorimeter expressed in L (luminance) values for all groups showed a decrease in first measure [26].

Melasma is a topical disorder manifesting a dark color distributed in spots in the skin. The treatment with pomegranate extract can demonstrate a visible amelioration, due the measures of luminescence showed in this experiment.

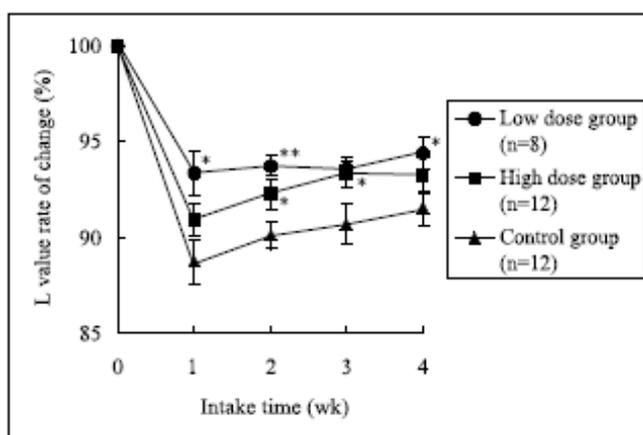


Fig 6. Change in L values in the UV-irradiated region on the inside of the upper arm in the three groups. Rate of change in L value at each measuring time point versus the baseline values considered

as 100% are shown. Data are means±SE *p<0.05 versus control group, **p<0.01 versus control group.

In only 1 week the test group showed an inhibition in melanin production up to 1.73% of inhibition compared to Placebo group.

Both, group high dose pomegranate extract and low dose show minimum pigmentation during the study (see Fig.6).

It is known that ellagic acid present in pomegranate extract inhibits melanin formation by acting on tyrosinase, which is the main enzyme-producing melanin.

Pomegranate extract orally administered to humans has a protective effect on slight sunburn caused by UV irradiation even at such low doses as were used in the study.

- **Evaluation of erythema and melanin**

The change in melanin value (measured with Mexameter) was evaluated in test groups. A tendency to inhibit the increase in melanin production was observed after 1 week of intake.

Changes in erythema were observed and measured. A tendency to inhibit the increase in the erythema values was observed after 1 week of intake.

- **Results of questionnaire**

The items answered concerning the state of skin conducted at the end of the test food intake period and were analyzed using Wilcoxon’s paired test, indicating improvement in “brightness of the face” and “stains and freckles” larger in test groups.

4.3.3. INGESTION OF VIQUA® SUPPLEMENT AND STUDY IN SKIN CONDITIONS

4.3.3.1. Study description

A placebo-controlled double-blind ingestion study of VIQUA® supplement was performed. A double blind, placebo controlled clinical study on 30 volunteers with orally administered. Clinical trial was

realized in base on the dose for the effect achievement 250 mg/day (125 mg twice a day).

The evaluated parameters were: Hydration, Tonicity, Inflammation, Desquamation, and Elasticity. The subjects were asked to keep in the rest in the room for at least 30 minutes. Further, with respect to the make up at the measurement site, it was in principle prohibited to put on makeup from 60 minutes before the inspection.

During the study, subjects were asked to give a self-assessment of their visual skin appearance using a Visual Numerical Scale (0-10).

4.3.3.2. Method

The study was a placebo-controlled double blind study of 4 weeks ingestion of VIQUA® supplement containing pomegranate extract and Placebo (same appearance of test supplement). The ingestion period was 4 weeks in both groups.

The treatment consisted in 2 tablet of 125 mg (product or placebo) taken twice a day, so the total diary intake is 250 mg orally per day.

4.3.3.3. Instruments used

*Corneometer CM 825 - Visioscan VC 98 – Skicon

Several parameters related to skin have been monitored using the face as test area.

4.3.3.4. Subjects selection

Individuals who showed signs of skin ageing (facial lines, wrinkles, dark spots, loss of skin elasticity) were recruited. 30 individuals were evaluated (female: 30 – Between 30 and 55 years) skin phototype I-IV. Before execution of the study, physicians fully explained the purpose of this study to the subjects, and written consents were obtained.

The subjects treated with VIQUA® always expressed a higher satisfaction score compared with those who were given the placebo Pomegranates are nature's secret gift to youthful, beautiful looking skin.

VIQUA® follows where nature leads, in this case powered by the innovative ADS nano-delivery system made of pomegranate itself, VIQUA® concentrates the beautifying essence of pomegranate in its most powerful and bioavailable form.

The result is a whitening, anti-ageing and anti-inflammatory beauty product with clinically proven efficacy. Its perfect stability and safety allows use in a diverse range of food and cosmetic applications.

This new generation nano delivery system demonstrates how advancement in nanotechnology exploits the best of nature and science to raise the efficacy and safety of active ingredients to a new level.

4.3.3.5. Variables assessed

- **Skin hydration:**

Skin hydration improved significantly under treatment, whereas the placebo group showed a decrease.

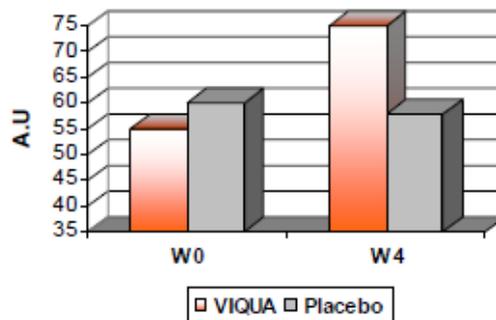


Fig 7. Comparing skin hydration condition before and after applying Viqua / Placebo.

Re-establish the natural healthy balance of the skin. Improve dry skin by boosting the health and strength of the layers of the skin so that it naturally keeps hydration locked in.

Skin hydration improved significantly under treatment (+40%), whereas the placebo group showed a decrease (-0,3%) (See Fig 7).

- **Skin roughness:**

The skin roughness, measured with Visioscan VC98, has significantly decreased only after the 0 active treatments.

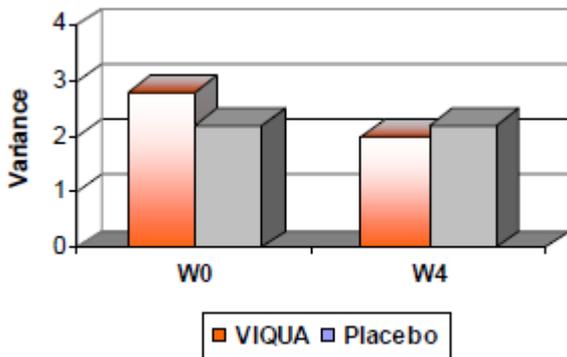


Fig 8. Comparing decrease of skin roughness before and after applying Viqua / Placebo.

The skin roughness, measured with Visioscan VC98, has significantly decreased only after the active treatment (-31%)(see Fig 8).

- **Skin smoothness:**

The surface is a parameter measured with Visioscan VC98, which calculates the size of the wavy skin surface, is an indirect measurement of skin smoothness. High smoothness will lead to decreased surface. In this case; the surface has significantly decreased only after the active treatment. The skin smoothness has improved only after the active treatment.

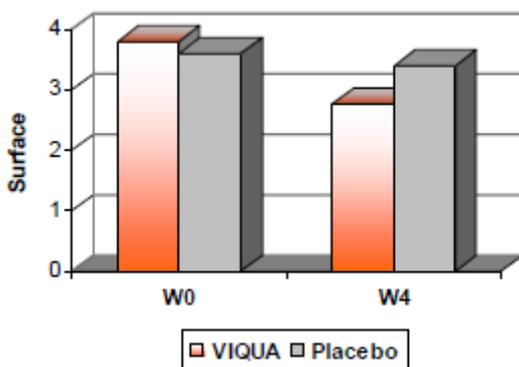


Fig 9. Comparing surface parameter of skin before and after applying Viqua / Placebo.

The active treatment increased the health and strength of the dermis, epidermis and supporting

structures, resulting in healthier, younger looking skin.

Skin smoothness improved, the surface parameter has significantly decreased only after the active treatment (-27%)(see Fig 9).

- **Depth of wrinkles:**

The volume parameter, measured with Visioscan VC98, is an indicator for the depth of wrinkles. In this study, the volume has significantly decreased only with the active treatment (see Fig 10).

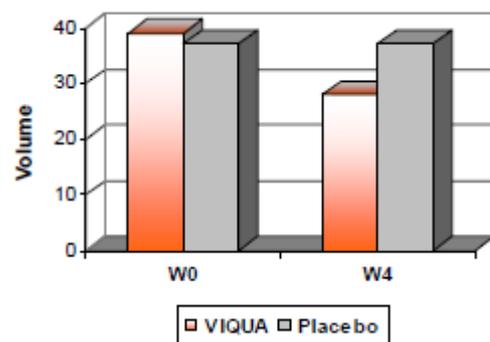


Fig 10. Comparing depth of fine lines and wrinkles before and after applying Viqua / Placebo.

A significant decrease in depth of fine lines and wrinkles was recorded (volume -26%).

- **Water content:**

The water content of stratum corneum, Measured by Skicon, has significantly increased only after treatment.

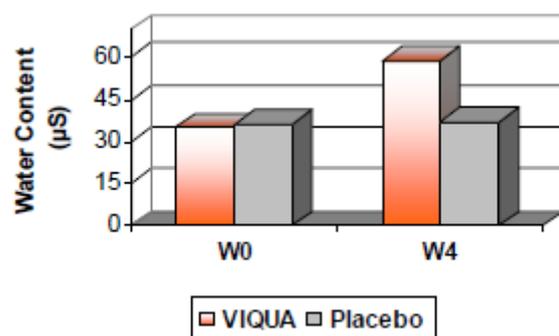


Fig 11.. Comparing water content of skin before and after applying Viqua / Placebo.

The water content of the skin's upper layers particularly improved with VIQUA® (51%)

Water content of stratum corneum has significantly increased only after the active treatment whereas in placebo group it is decreased. (see Fig 11).

- **Skin inflammation:**

Source of inflammation, dark spots have disappeared only after the active treatment. Skin inflammation has been significantly improved.

Source of inflammation, dark spots have disappeared only after the active treatment.

Significant inflammation inhibition has been noticed through blood vessels remodeling

- **Blood circulation:**

At Week0 it was observed high permeability of blood vessels.

Blood vessel remodeling suggested significant inhibition of inflammation processes.

At Week4 the permeability was significantly reduced and blood circulation increased [Annexe 1]

The self-assessment test shows evident perception's differences of all the estimated parameters that are correlated to the product's efficacy. The subjects assuming the active treatment have always expressed a higher score compared with the one's assuming the placebo.

The active treatment has proved much better than placebo. The clinical study confirms the results from the biophysical measurements.

The treatment was well tolerated and there were no reported side effects.

- **Action in Melasma**

Melasma (or chloasma) is a common disorder of cutaneous hyperpigmentation predominantly affecting sun-exposed areas in women. The pathogenesis of melasma is not fully understood and

treatments are frequently disappointing and often associated with side effects.

Melasma tends to appear during pregnancy (mask of pregnancy) and in women who take oral contraceptives, although it can occur in anyone. The disorder is most common in sunny climates and among people with darker skin. Irregular, patchy areas of dark colour appear on the skin, usually on both sides of the face. Rarely, melasma appears on the forearms.

In a clinical trial to test melasma evolution with antioxidants treatment has been realized. Factors like relationship to pregnancy, hormonal therapy, sun exposure and cosmetic use has to be taken. Patients are asked about their previous use of hydroquinones and family history of melasma.

Melasma may be due to blood circulation, which may cause the formation of patches.

For the melasma area, the diameter of the melasma area was measured by a ruler at three different places and the mean values were determined. The pigmentary intensity was rated using the national standard colour chart [27].

The benefits showed in melasma are:

Skin tone became generally brighter and dark spot/blemish color attenuated. The responsible are anthocyanins which can enhance blood circulation in cells signifying a Melasma reduction of 80%.

4.3.4. PHYTOESTROGENS IN POMEGRANATE

EXTRACT. ESTROGEN-LIKE BIOLOGICAL ACTIVITY

Japanese women are reported to have a low frequency of hot flushes compared with postmenopausal western women, in part attributed to their high phytoestrogen consumption. Whether this is valid has been recently challenged.

Evidence from several human studies demonstrates that certain dietary phytoestrogens can produce mild estrogenic effects in the postmenopausal woman, including estrogen-like effects on vaginal cytology