A Malus Domestica plant active providing soothing effect through decreasing inflammation and regulating cutaneous microcirculation

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Abstract

Sensitive skins always feel aggressed, so it is necessary to better regulate several mechanisms of inflammation. For skin in harmony with its environment, soothed, and with a tone more radiant and even. Malus Domestica plant active is an advanced product industrialized newly from plant cells that were dedifferentiated using their fruit cells and elicitated to produce a large quantity of phytoalexins.

In order to study the smothing effect of the Malus Domestica plant active, the tests on SKINETHIC® reconstituted epidermis and endothelial cells were conducted to prove its inhibitory effect on inflammatory mediators and vasodilators, and a clinical study was adapted to prove its lightening, soothing and anti-redness effects.

The study showed that the Malus Domestica plant active presents a significant inhibition effect on the inflammation mediators after induction by UVB rays.Additionally, the irradiation of the endothelial cells in culture with UVB results in an increase of nitric oxide. The treatment with the Malus Domestica plant active before irradiation of the cells results in a significant decrease of the nitric oxide release. The results obtained by clinical tests showed a decrease of the redness and irritation by 16%, 29%, respectively, on 20 women who use the emulsion with 0.1% of Malus Domestica plant active for 28 days.

In general, dedifferentiated and elicitated Malus Domestica plant active displays strong anti-inflammatory and anti-redness properties by the inhibition of inflammatory mediators and vasodilators.

Key words: active plant cells; inflammation; microcirculation; soothing

Introduction

Epidemiological studies have shown a high prevalence of sensitive skin over the world with 60-70% in women and 50-60% in men. [1-2] Among them, the skin of Caucasians is more susceptible to sensitivity, which may be related to the difference of skin melanin content in different races; In addition, sensitive skin is also more likely to occur in young people, as a result of that the reduction of nerve distribution on the skin surface in old people can cause decreased sensory nerve function. [3-4] There are many factors lead to sensitive skin: 1. Environmental (atmospheric pollution, seasonal changing, UV irradiation, air humidity, etc.); 2. Chemical stimuli (cosmetics, hair dyes, detergent, etc.); 3. Lifestyle (alcohol, coffee, stay up , diet, stress, etc.); [5-7] 4. Sensitive skin may also be hereditary in patient's family or some sensitive skin patients with certain skin diseases(such as dermatitis, psoriasis, rosacea, etc.) are hypersensitivity to external stimuli.[8-9]

The occurrence of sensitive skin is always accompanied with unpleasant sensations, such as, tightness, pruritus, tingling and skin redness, which is more sensitive and responsive to external stimuli than normal skin.[10] The pathophysiology of sensitive skin may be related to three main hypotheses which are impaired skin barrier function, increased nerve conduction function and increased inflammatory response. [11] When the skin barrier is disrupted, it will accelerate trans-epidermal water loss and nutrient loss, which will decrease the content of lipids and sphingolipids in the skin, and enhanced the permeability of irritants or allergies , so that the nerve conduction function will increase without adequately protected, and the inflammatory immune system response in skin will aggravate alone with symptoms such as tightness, pruritus, tingling and skin redness.[12-14]

External stimuli can elicit a stronger immune response in sensitive skin. When exposed to the stimuli, the keratinocyte will secrete a large number of inflammatory mediator to activate more advanced immune cells (macrophages, lymphocytes, mast cells, T cells, granulocytes) into a fighting state, which can kill pathogenic bacteria or release inflammatory mediator to further trigger a stronger inflammatory response.[15-16] Inflammatory mediators are chemicals produced in cells and body fluids during inflammation that cause an inflammatory response, of which the common inflammatory mediators associated with sensitive skin are interleukin-1α(IL-1α),interleukin-6(IL-6), prostaglandin E2 (PGE2),nitric oxide (NO). IL-6 is a inflammatory mediators secreted by T cells, B cells, monocytes, and endothelial cells after the body is stimulated by inflammation. IL-6 can active vascular endothelial cells and inflammatory cells, and more importantly, it can induce the synthesis of acute phase protein,

catalyze and amplify the inflammatory response and toxic effects. Overexpression and release of IL-6 can cause damage to skin tissue.[17]IL-1α is an intracellular messenger cytokine synthesized then stocked inside the cell as an inactive precursor, and mediate cellular responses through autocrine and paracrine. In response to certain stimuli, IL1-αshow a high expression in topical sensitive skin. IL1-αbinds to the receptor and activate the release of other inflammatory mediators (such as: IL-6), what's more a large amount of IL-1α secretion can lead skin to become hot and red.[18-19]Studies have shown that PGE2 is one of the important inflammatory mediator in the inflammatory cascade, involved in inflammation through activating 4 different G protein coupled receptors with symptoms of pain, warm, swelling, and redness. COX-2 overexpression can convert arachidonic acid into PEG2 and then PEG2 can also induce macrophages to release inflammatory mediators IL-1, IL-6 to further aggravate the inflammatory response. [20-21] Nitric oxide (NO) is a gas of small molecule with toxic properties, but it is also a biologically active substance involved in numerous pathologic and physiologic processes. NO shows strong antagonistic action against sympathetic nervous excitement, which mediates smooth muscle relaxation in blood vessels. External stimulation will increase the release of NO to cause vasodilation, which makes inflammatory factors are more likely to enter the blood vessels to aggravate skin sensitivity, and even cause allergies. [22]

Recently, the occurrence of sensitive skin for people has a growing trend. With the increasing of the concern of skin health and aesthetic standards, people are more inclined to use allergy-releasing cosmetics to improve sensitive skin. At present, many cosmetic components have the effect of effectively inhibiting the inflammatory response through decreasing the expression of inflammatory mediators to relieve the symptoms of tightness, pruritus, tingling and skin redness. Apple contains an extremely useful active element known as polyphenol with excellent anti-inflammatory and anti-oxidant effect, the main components of which include chlorogenic acid, epicatechin, apple condensation tannins, phlorizin, anthocyanins, etc. Zessner et al. demonstrated that epicatechin and phlorizin are COX-2 inhibitors that can reduce the production of PGE2 [23]; Shan et al. found that chlorogenic acid decrease the expression of COX-2 protein by inhibiting the activity of NF-κB and JNK/AP-1. [24] The composition of apple extracts from a variety of sources has different effect, and their anti-inflammatory activity and mechanism remain to be investigated.

Increasing emphasis given to the allergy-relieving products, the cosmetics industry

continually develop new active composition for sensitive skin. This thesis is concerned with the effect of Malus Domestica plant active obtained from the plant cell cultivation technology.

Materials and Methods

1.Plant Cell culture protocol

Malus Domestica fruit were surface-sterilized and were each cut into small pieces, wounded, and placed on solid medium. Cell suspension cultures of Malus Domestica were established from fruit callus and maintained under continuous fluorescent light (5000 lux) at 25±1°C in Erlenmeyer flasks containing cell suspension on an orbital shaker. The maintenance medium contained macro-elements, microelements and vitamins and was supplemented with sucrose, 1-naphtalene-acetic acid, and kinetin. Cells were sub-cultured every week by inoculating the cells at a 1/5 (v/v) ratio into fresh medium. The obtained cells were elicited and used for the different studies after extract preparation.

2.Principle of culture

2.1 endothelial cells (HUVEC)

The endothelial cells were distributed in multiwell boxes at a rate of $2x10^5$ per well (96 wells) in 0.2 ml of culture medium. They were then maintained for 24 hours in an incubator with (5% of CO_2). The product was distributed at various concentrations in the multiwell plates at a rate of 8 wells per dose. The time of contact of the cells with the the Malus Domestica plant active was 24 hours at 37°C.

2.2 Human reconstituted epidermis

Keratinocytes of human origin were sown on 0.5cm² polycarbonate filters in a defined medium (MCDB 153 modified) and supplemented. The cells were cultured for 14 days at the air/liquid interface, and the culture medium was changed every two days. The epidermises thus formed were used for the study completion since the 14th day of the culture. The time of contact of the epidermises with the Malus Domestica plant active was 24 hours.

3.Cutaneous microcirculation regulation assay

The endothelial cells were treated for 24 hours, then 75 µl culture medium were collected and mixed with an equal volume (75µl) of Greiss reagent (1% sulfanilamide; naphthylene diamine 0.1% and 2%phosphoric acid). Solutes were incubated 10 minutes at room temperature. The product of the reaction was assayed using a plate reader at a wavelength of 450 nm. The reference range was carried out using sodium nitrate (1.5; 10 and 20 µM). This experience

allows the assessment of the activity of the constitutive NO synthase. The assay of NO, which has enabled us to assay the activity of the induced NO synthase was performed in the same way. The only difference lies in the induction of the enzyme by irradiation with ultraviolet rays.

4.Anti-inflammatory assessment

24 hours after the treatment of reconstituted epidermises;the culture mediums were taken, and the assessment of inflammatory mediators was performed according to the protocols described in the Interleukin 1- (IL1-α) kit、 Interleukin 6 (IL-6) kit and Prostaglandin E2 (PGE2) kit.

5.Clinical test

The emulsion with 0.1% of Malus Domestica plant active (powder form) was applied by 20 volunteers(44-67 years old women) who have sensitive and reactive skin for 28±2 days under the normal conditions of employment, and the colorimetric measurement, sting test, and questionnaire were conducted during two visits at the laboratory (D0 and D28).

5.1 Assessment of the anti-redness effect by colorimetric measurements

The color of the skin on the cheeks was assessed by colorimetric measurements (CL400®) at each experimental time. The probe of the colorimeter ® CL400 sends white light from LEDs and illuminates the skin in circular and uniform manner. The emitted light is dispersed in all directions, some through the layers of the epidermis and the other part is disseminated outside of the skin. The light reflected from the skin is measured by the probe.The measurement of skin color is presented under the coordinates model L * a * b * .The L* component is clarity, which ranges from 0 (black) to 100 (white). The component a* represents the range of red axis (positive value) → green (negative) through the white (0) if clarity is 100. The b* represents the range of yellow axis (positive value) → blue (negative) through the white (0) if clarity is 100. The anti-redness effect was obtained by comparing the values characterizing the color of the skin measured before (D0/T0) and after 28 days (D28) treatment.

5.2 Assessment of the soothing effect by stinging test

The determination of skin reactivity of each volunteer was conducted at the inclusion by a stinging test. The investigator wipes the nasolabial furrow of the volunteer with cotton. A solution of lactic acid (10%) is applied by five passages using a cotton swab at the nasolabial

furrow compared to physiological serum applied simultaneously to the other side. This test based on subjective self-assessment by the volunteer of the burning and tingling felt, after the application of two solutions. 5 minutes after the application of the solutions, the investigator wipes the nasolabial furrow of the volunteer with cotton moistened with distilled water. The soothing effect was determined by a stinging test performed 10 minutes after product application and after 28 days of application under normal conditions of use on volunteers initially declared responsive.

5.3 Assessment of the effect on the cutaneous state by self-scoring

An improvement of the cutaneous state by self-scoring after 28 days of applications in conditions of normal use is studied on female of Phototype (Fitzpatrick, from Ⅱ to III) with sensitive and reactive skin aged from 44 to 67 years old and of all skin types on the face presenting with redness on the face. The self-scoring consists in a self-assessment of the cutaneous state before then after application of the investigational product to the experimental area, in order to determine its cosmetic efficacy. For this purpose, The volunteers answered on D28 to a multiple choice questionnaire which gathered items concerning the product “Malus Domestica plant active ” Items n°1 to 19: the cosmetic qualities and performances of the product, following an ordinal scale in 4 points established with the Promoter *(agree, almost agree, almost not agree, not agree).*

Results

1.Cutaneous microcirculation regulation assay

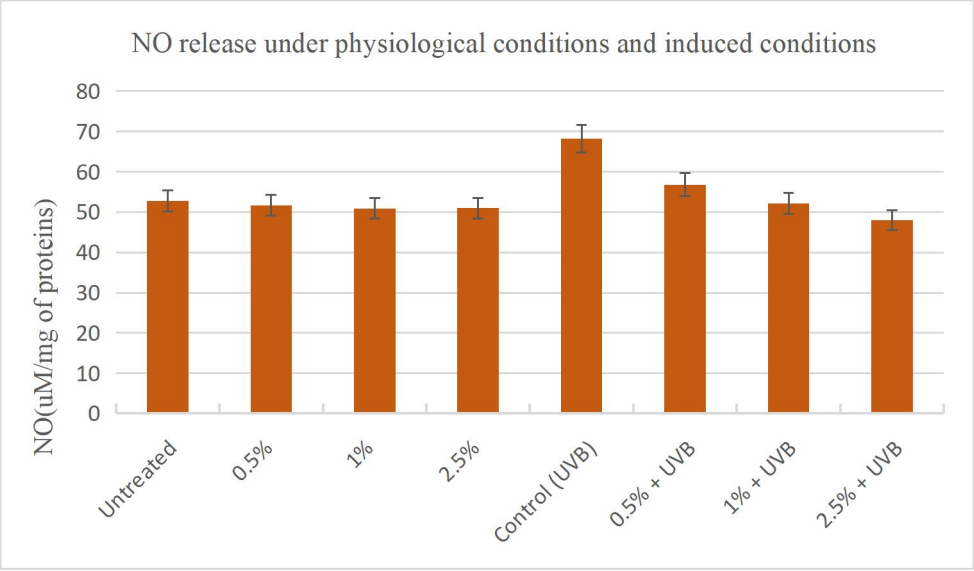

Figure 1：NO release under physiological conditions and induced conditions（UVB）after treatment of Malus Domestica plant active.

The obtained results show that the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% has no effect on the Nitric oxide release under physiological conditions.And the irradiation of the endothelial cells in culture with ultraviolet rays (150mJ/cm²) results in an increase of nitric oxide (+29%). The treatment with the Malus Domestica plant active at (0.5%, 1% and 2.5%) prior to irradiation of the cells results in a significant decrease of the nitric oxide release by 17%, 24% and 30%.

2.Anti-inflammatory assessment

2.1.-Interleukin 1-α assessment under physiological conditions

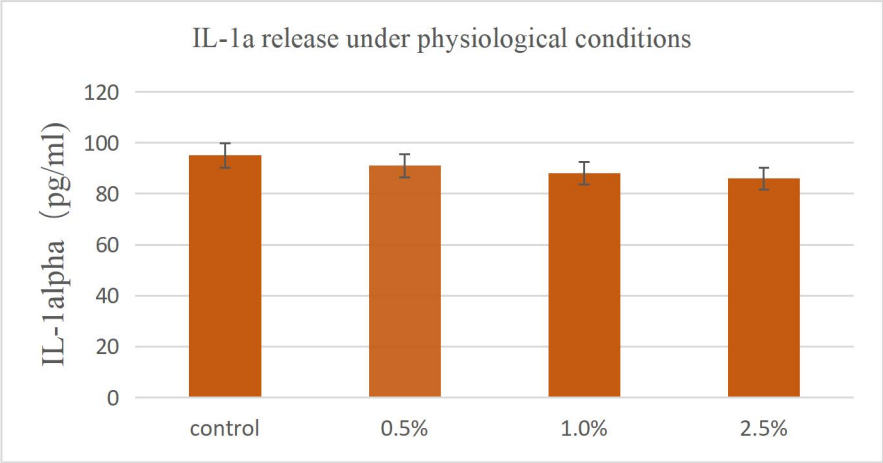

Figure 2： IL-1 α release under physiological conditions after treatment of Malus Domestica plant active.

The obtained results show that the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% has no effect on the Interleukin 1-α (IL1-α) release under physiological conditions.

2.2.-Interleukin 1-α assessment under induced conditions by UVB irradiation

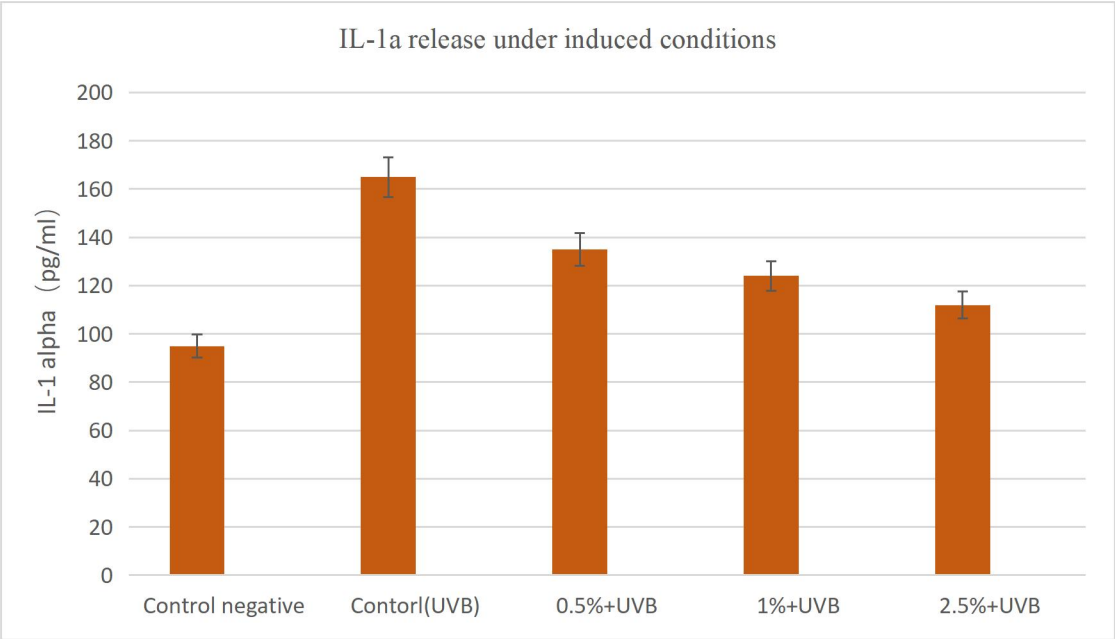

Figure 3：IL-1α release under UVB irradiation conditions after treatment of Malus Domestica plant active.

The results demonstrate that Interleukin 1-α release was increased by the UVB compared to the negative control. This release was inhibited by the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% respectively by 18%, 25% and 32%.

2.3 Interleukin 6 assessment under physiological conditions

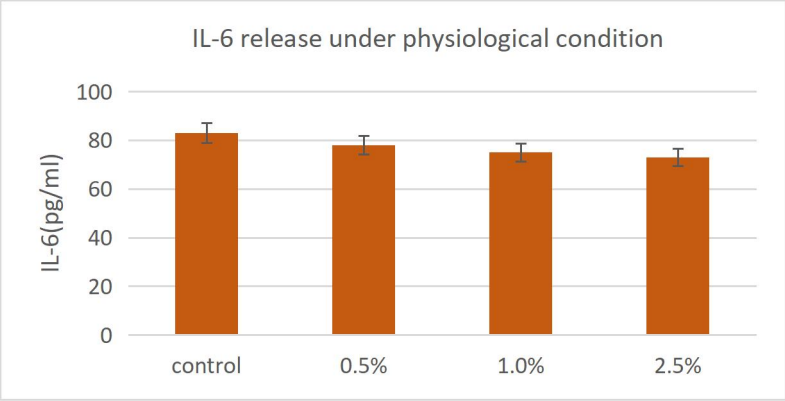

Figure 4：IL-6 release under physiological conditions after treatment of Malus Domestica plant active.

The obtained results show that the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% has no effect on the Interleukin 6 (IL-6) release under physiological conditions.

2.4 Interleukin 6 assessment under induced conditions by UVB irradiation

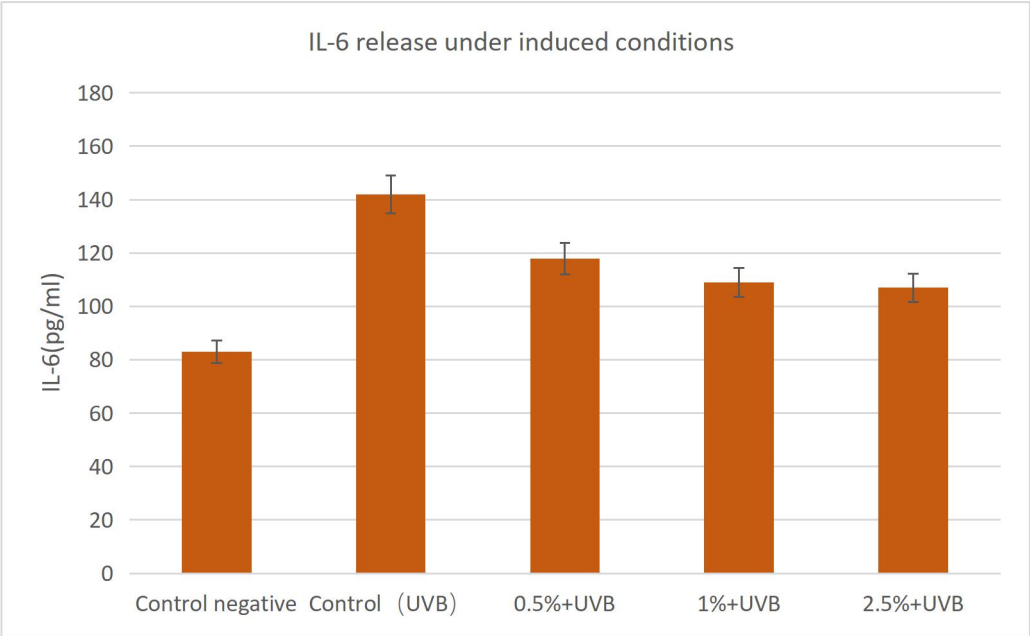

Figure 5：IL-6 release under UVB irradiation conditions after treatment of Malus Domestica plant active.

The results demonstrate that Interleukin 6 (IL-6) release was increased by the UVB compared to the negative control. This release was inhibited by the Malus Domestica plant active at the concentrations of 0.5%, 1.0% and 2.5% respectively by 17%, 23% and 25%.

2.5 Prostaglandin E2 (PGE2) assessment under physiological conditions

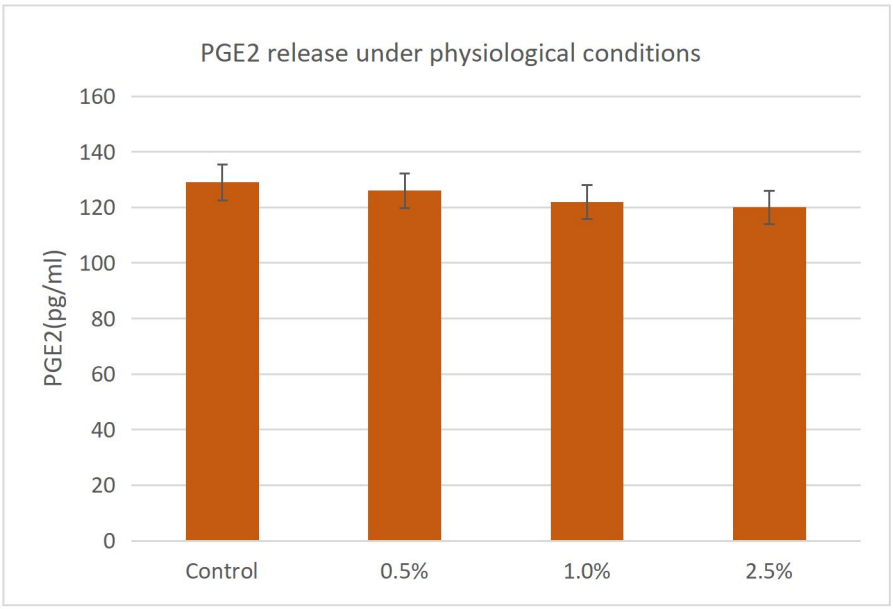

Figure 6：PGE2 release under physiological conditions after treatment of Malus Domestica plant active.

The obtained results show that the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% has no effect on the Prostaglandin E2 (PGE2) release under physiological conditions.

2.6 Prostaglandin E2 (PGE2) assessment under induced conditions by UVB irradiation

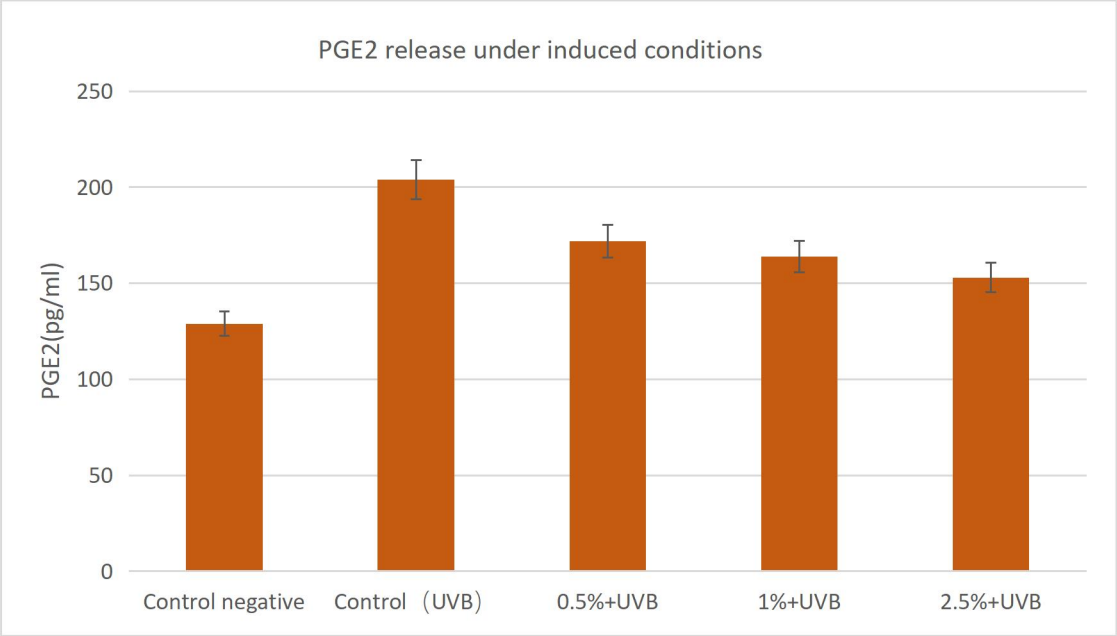

Figure 7：PGE2 release under UVB irradiation conditions after treatment of Malus Domestica plant active.

The results demonstrate that Prostaglandin E2 (PGE2) release was increased by the UVB compared to the negative control. This release was inhibited by the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% respectively by 16%, 20% and 25%.

3.Clinical test

3.1 Assessment of the anti-redness effect by colorimetric measurements

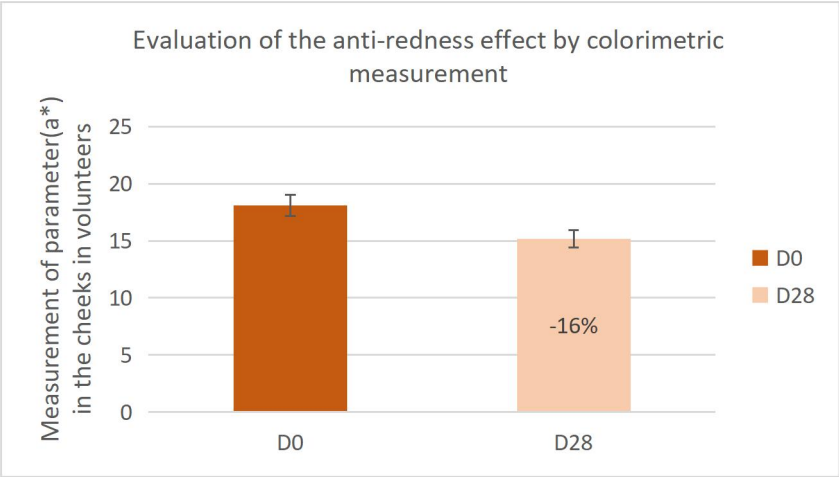

Figure 8：anti-redness effect by colorimetric

The results shows a decrease of the redness after 28 days of application of the Malus Domestica plant active by 16% as compared to day 0.

3.2 Assessment of the soothing effect by stinging test.

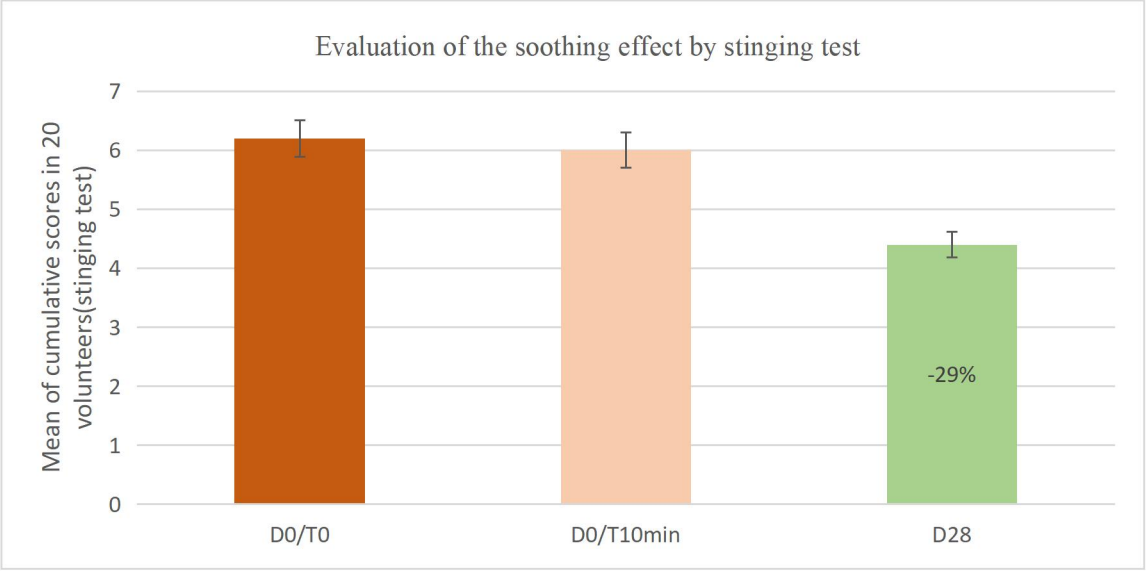

Figure 9： soothing effect by stinging test

The results shows a decrease of the irritation after 28 days of the Malus Domestica plant active by 29% as compared to day 0. Skin is effectively soothed.

3.3 Self-assessment of the cosmetic qualities and performances.

N°	Items	% D28
1	The application is easy	90%

2	The consistency is just right	95%
3	The product is not fat	90%
4	The product is not sticky	85%
5	The product penetrates well	95%
6	The smell is pleasant	90%
7	The fragrance is just right	95%
8	The color is nice	90%
9	The texture is nice	100%
10	The skin is hydrated	100%
11	The skin is comfortable	95%
12	The skin is soft	95%
13	The skin is supple	95%
14	The skin is soothed	90%
15	The redness are reduced	85%
16	The skin is less sensitive	80%
17	The skin reactions (redness) are visibly reduced	75%
18	Feelings of discomfort are diminished	80%
19	The product satisfied you?	90%

Table:Volunteers' Self-Assessment

All the volunteers declared that the skin is hydrated and the texture is nice. 90% of the volunteers declared that the skin is soothed. 85% of the volunteers declared that the redness is reduced. 80% of the volunteers declared that feelings of discomfort are diminished 75% of the volunteers considered that the skin reactions (redness) are visibly reduced 90% of the volunteers declared being satisfied by the product.

Discussion

In the present experimental conditions, the product Malus Domestica plant active cells applied on reconstituted epidermis and on face for 28 days of application showed that relevant effects on：

- **Cutaneous microcirculation regulation**

The assessments of nitric oxide demonstrate that the Malus Domestica plant active at the concentrations of 0.5%, 1% and 2.5% reduces significantly nitric oxide production induced by UVB. The Malus Domestica plant active could eliminate the vasodilator effect of nitric oxide (EDRF: endothelium derived relaxing factor) after ultraviolet irradiation. Indeed, nitric oxide released after UVB irradiation can react with superoxide anion (O^{2-}) to form peroxynitrite ($ONOO^{-}$), which are unstable anion and can damage the endothelial cells and therefore cutaneous microcirculation.

- **Anti-inflammatory effect**

The Malus Domestica plant active (0.5%, 1% and 2.5%) exerts a significant inhibitory action against irritative and inflammatory phenomenon ' s induced in vitro on reconstituted epidermises.The Malus Domestica plant active protect the skin from ultraviolet irradiation and damage caused by external stimuli by limiting the release of inflammatory factors(IL-1,IL-6,PGE2).

- **Clinical assessment**

The Malus Domestica plant active soothes and enhances the general well-being of the skin. The results obtained by clinical test showed an decrease of redness and irritation by 16% and 29% respectively after 28 days of application in women with sensitive and reactive skin.

Acknowledgments.
NONE.

Conflict of Interest Statement.
We have no conflicts of interest to declare.

References

1. Do L H D, Azizi N, Maibach H (2020) Sensitive skin syndrome: an update.American journal of clinical dermatology 21(3): 401-409.

2. Brenaut E, Barnetche T, Le Gall-Ianotto C, et al (2020) Triggering factors in sensitive skin from the worldwide patients' point of view: a systematic literature review and meta-analysis.Journal of the European Academy of Dermatology and Venereology 34(2): 230-238.

3. Sensitive skin syndrome[M]. CRC Press, 2017.

4. Kamide R, Misery L, PEREZ-CULLELL N, et al (2013) Sensitive skin evaluation in the J apanese population. The Journal of dermatology 40(3): 177-181.

5. Berardesca E, Farage M, Maibach H (2013) Sensitive skin: an overview. International journal of cosmetic science 35(1): 2-8.

6. Farage M A (2019) The prevalence of sensitive skin. Frontiers in medicine 6: 98.

7. Misery L, Myon E, Martin N, et al (2007) Sensitive skin: psychological effects and seasonal changes. Journal of the European Academy of Dermatology and Venereology 21(5): 620-628.

8. Mikkelsen C S, Holmgren H R, Kjellman P, et al (2016) Rosacea: a clinical review. Dermatology reports 8(1).

9. Willis C M, Shaw S, De Lacharriere O, et al (2001) Sensitive skin: an epidemiological study. British Journal of Dermatology 145(2): 258-263.

10. Misery L, Loser K, Ständer S (2016) Sensitive skin. Journal of the European Academy of Dermatology and Venereology 30: 2-8.

11. Handbook of cosmetic science and technology[M]. CRC press, 2014.

12. Frosch P (1977) Method for appraising the stinging capacity of topically applied substances. J Soc Cosmet Chemist 28: 197-209.

13. FAN L, HE C, JIANG L, et al (2016) Brief analysis of causes of sensitive skin and advances in evaluation of anti-allergic activity of cosmetic products. International Journal of Cosmetic Science 38(2): 120-127.

14. CHO HJ, CHUNG BY, LEE HB, et al (2012) Quantitative study of stratum corneum ceramides contents in patients with sensitive skin . Dermatol 39(3): 295-300.

15. Rerknimitr P, Otsuka A, Nakashima C, et al (2017) The etiopathogenesis of atopic dermatitis: barrier disruption, immunological derangement, and pruritus. Inflammation and regeneration 37(1): 1-15.

16. Talagas M, Misery L (2019) Role of keratinocytes in sensitive skin. Frontiers in Medicine 6: 108.

17. Haupt W, Fritzsche H, Hohenberger W, et al (1996) Selective cytokine release induced by serum and separated plasma from septic patients. The European journal of surgery= Acta chirurgica 162(10): 769-776.

18. Kaneko N, Kurata M, Yamamoto T, et al (2019) The role of interleukin-1 in general pathology. Inflammation and Regeneration 39(1): 1-16.

19. Dinarello C A (2018) Overview of the IL-1 family in innate inflammation and acquired immunity. Immunological reviews 281(1): 8-27.

20. FESTA-VASCONCELLOS J S, PIRANDA D N, AMARAL L M, et al (2012) Polymorphisms in cycloxygenase-2 gene and breast cancer prognosis: association between PTGS2 haplotypes and histopathological features . Breast Cancer Research & Treatment 132(1): 251-8

21. IBRAHIM A S, SYAED K W, MOHAMED E T M, et al (2012) In vitro and in vivo anti-inflammatory activities of columbin through the inhibition of cycloxygenase-2 and nitric oxide but not the suppression of NF-κB translocation. European Journal of Pharmacology 678(1-3): 61-70.

22. Lee R H, Efron D, Tantry U, et al (2001) Nitric oxide in the healing wound: a time-course study. Journal of Surgical Research 101(1): 104-108.

23. Zessner H, Pan L, Will F, et al (2008) Fractionation of polyphenol-enriched apple juice extracts to identify constituents with cancer chemopreventive potential. Molecular nutrition & food research 52(S1): S28-S44.

24. Shan J, Fu J, Zhao Z, et al (2009) Chlorogenic acid inhibits lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264. 7 cells through suppressing NF-κB and JNK/AP-1 activation. International immunopharmacology 9(9): 1042-1048.