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Sensitive skin: R&D approach to substantiate the efficacy of a promising nutricosmetic ingredient to alleviate skin sensitivity

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1. Introduction

Sensitive skin (SS) affects approximately 60% of the population, regardless of gender, skin type, or phototype [1]. The International Forum for the Study of Itch defined SS as the occurrence of unpleasant sensations (tingling, burning, pain, itching, prickling) in response to stimuli that, normally, should not cause such sensations. These unpleasant sensations cannot be explained by lesions attributable to a specific skin disease. The skin may appear normal or may be accompanied by erythema. Sensitive skin can affect any area of the skin, with the face being particularly prone [1] [2]. Moreover, SS causes stress and discomfort, thus impacting overall quality of life. Various external factors (such as wind, cold, pollution, or unsuitable skin-care products) and internal factors (such as stress, emotions, and hormonal fluctuations) can trigger skin sensitivity. The relationship between skin sensitivity, sensory processes, and emotions is complex and interconnected. Sensory receptors, such as TRPV1, respond to stimuli like pain or temperature. In sensitive skin, these receptors can become hyperactive, intensifying sensations. Additionally, emotional states like stress or anxiety can worsen sensitivity by triggering hormonal changes that could impact the skin barrier, making it more prone to irritation and external aggressions [3] [4].

WLE is a lipidic extract derived from the endosperm of wheat grain (*Triticum aestivum*), rich in polar lipids such as phospholipids, glycolipids (inclunduing digalactosyldiglycerides), and sphingolipids (including glycosylceramides and ceramides). The hydrating and anti-aging efficacy of a daily supplementation of 350 mg of WLE has previously been demonstrated in subjects with dry skin. WLE polar lipids play a crucial role in maintaining skin barrier integrity by limiting water loss from the epidermis. Based on these data, it was hypothesized that WLE would benefit sensitive skin.

In this research work, the scientific approach to substantiate the efficacy of a promising nutricosmetic ingredient to alleviate skin sensitivity is described. First, a transcriptomic study on skin explants was set up to determine the impact of WLE at the mRNA levels. The modulation of CNR2 and HAS2 was subsequently confirmed at the protein level using immunofluorescence techniques. Furthermore, a study using an *ex vivo* induced SS (ISS) model analyzed the effect of WLE on TRPV1 receptors, IL8, mast cells degranulation and histamine levels. Finally, a three-month oral supplementation clinical study measured the transepidermal water loss (TEWL) and redness scores in subjects with SS supplemented with WLE versus placebo.

2. Materials and Methods

1- Raw material/Tested product

WLE was obtained according to a patented manufacturing process.

2- *Ex vivo* investigation

2.1- Skin explants

The skin explants were obtained from patients undergoing plastic procedures in compliance with French law L.1245 CSP “Products and elements of the human body taken during surgical procedures and used for scientific research”. Each patient was fully informed and provided with written consent prior to donating their tissue. For transcriptomic analysis, abdomen skin samples from seven skin samples from Caucasian donors were supplied by Biopredic (Saint-Grégoire, France). For the ISS model evaluation, abdomen, breast and arms skin samples from eight skin samples from Caucasian donors were supplied by Hôpital Universitaire Pitié-Salpêtrière (Paris, France).

2.2- Tissue culture and treatment

For transcriptomic analysis, explants from seven donors were placed on day 0 in DMEM medium at 37°C, in a humid atmosphere, enriched with 5% of CO2. The explants were divided into three experimental groups, each with three replicates (n = 3). WLE was diluted in DMSO and added to the medium at 6.7 µg/ml for systemic application. The explants were treated once daily for 3 days, with the control group receiving no treatment except for medium renewal. On day 4, the explants of each batch were collected, cut in half where one part was used for RNA extraction and PCR analysis, while the other was included in Optimum Cutting Temperature gel (OCT) and stored at -80°C until further analysis.

In the ISS model assay, the explants were from eight donors. At T0, two hours before the sensitivity induction, 6.7 µg/ml of WLE were applied in the culture medium to mimic a systemic intake. As WLE was diluted in DMSO for the assay, a similar dilution of DMSO was used as a control.

The ISS model was set up by a topical application of 15 µL/cm^2 of 1% Sodium Lauryl Sulfate (SLS) for 1 hour then rinse in PBS. This was followed by a UVB session at 2J/cm^2 using a Vilber Lourmat lamp. Finally, 5 µM capsaicin (CAP) was added to the culture medium. On day 1, the culture medium was renewed, and the products were reapplied systemically for an additional 24 hours.

On day 2, the skin fragments were removed and fixed in formalin for analysis of TRPV1 and mast cells. The culture supernatants were collected and frozen at -32°C for IL8 and Histamine analysis.

2.3- From Gene Expression to Protein: A Comprehensive Analysis

2.3.1- LDH Detection Assay

The LDH detection assay was performed on the culture medium at the end of the treatment. A commercial LDH assay kit was procured (Thermo Fisher Scientific, Waltham, MA, USA) and assays were performed in accordance with the manufacturer's instructions. A negative control (blank) was done using fresh culture medium without tissue. Optical densities from the blank were used to normalize those obtained from other samples. A positive control test was performed using Triton 0.1%, which yielded LDH release with an optical density 10-fold higher than any other test sample.

2.3.2- Extraction and expression of CNR2 and HAS2 mRNA by RT-qPCR method

Total RNA from samples were homogenized in Trizol reagent (Invitrogen, TRIzol Plus RNA purification kit). RNA was extracted following the manufacturer's instructions. The quality controls were performed using Agilent RNA pico kit (Agilent, 5067-1513) with Analysis Agilent 2100 bioanalyzer. The total RNA quantitation was performed using a nanodrop spectrophotometer. For CNR2 and HAS2 quantification, mRNA was reverse-transcribed into cDNA with Superscript VILO cDNA Synthesis Kit (Invitrogen, 11754050). Quantitative PCR was performed with a Platinum Quantitative PCR SuperMix-UDG Kit (Invitrogen, 11733046) using the CFX-connect (Biorad). The results were normalized to endogenous control GAPDH expression and expression level of mRNA target was calculated using 2ΔΔCt method.

2.3.3- Fluorescence-based quantification of CB2 protein and hyaluronan (HA)

The frozen block, containing the biopsy, was placed in a cryostat cabinet (Leica CM3050 S) and sectioned at a thickness of 8µm. Sections were mounted on polylysine superfrost slides (Epredia, J2800AMNZ). The slides were stored at -80°C prior to staining. Slides of 3 skin donors were thawed for 5 minutes and then fixed in Formalin for 15 minutes. They were incubated in TBS-T 5% BSA at room temperature for 1 hour and incubated overnight at 4°C with a specific primary antibody for CB2 (Abcam, ab3561) or bHABP (Sigma-Aldrich, 385911-50UG). The slides were then incubated either with secondary antibody anti-mouse Cyanine 5 (Jackson Lab, 115-175-166) for CB2 or with with Streptavidine, conjugated Alexa Fluor™647 (Thermofisher, S32357) for 1h at room temperature in the dark. Finally, the slides were incubated with Hoechst® (Thermo fisher Scientific, 33342) for 10 minutes and preserved in mounting medium Fluoromount-G (Thermo Fisher Scientific, 00-4958-02). Each step was followed by three washes with TBS-T for 5 minutes. The images were captured using an epifluorescence microscope (Zeiss, Axio Imager Z1, ApoTome, Zen2 blue edition software) equipped with Cyanine 5 and AlexaFluor 647 channels and analyzed with a deep learning algorithm developed internally.

2.4- Induced Sensitive skin model

2.4.1. Immunohistochemical analysis of TRPV1 receptors

A vanilloid R1/TRPV1 antibody (Novusbio, polyclonal rabbit antibody, IgG) was used. Immunodetection was performed using an indirect immunoperoxidase technique in two levels, amplified with the Polink-2 Plus kit (GBI) and stained in red using 3-amino-9-ethylcarbazole.

Semi-quantitative scoring was employed to evaluate the immunostaining intensity, with a score of 0 indicating negative staining and a score of 4 representing very strong staining in the granular and superficial layers of the epidermis. Scoring was conducted across the entire section, examining 10 to 15 fields at x400 magnification.

2.4.2. Mast cells histological analysis

Mast cells were stained with toluidine blue. They were then counted under the light microscope (approximately 15 fields at x 40 magnification) and classified using the following 3 scores:
- score 1: degranulated mast cells containing a low number of basophilic granules,
- score 2: mast cells containing a moderate number of basophilic granules,
- score 3: mast cells containing many basophilic granules.

For each skin analyzed, the percentage of mast cells of each score was calculated in relation to the total number of mast cells.

2.4.3. Enzyme-Linked Immunosorbent Assay

IL8 and histamine levels in culture media supernatants were assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) with spectrophotometric readings at 450 nm to determine concentrations (pg/mL).

The IL8 Biotechne Quantikine assay kit (Minneapolis, USA) and the Elabscience histamine kit (Houston, USA) were used. Since the skin fragments had the same surface area (1 cm^2), the results were normalized to the weight of each skin fragment (mg) and expressed as pg/mg.

3- Clinical Study

The clinical study aimed to evaluate the potential soothing effect of a three-month daily supplementation with WLE on a population with SS. This randomized, monocentric, double-blind, placebo-controlled clinical trial took place from August 2023 to December 2023 at Gredeco, France. The 60 subjects were assigned into two groups of 30 each. One group took a daily supplementation with 350 mg of WLE supplement, and the other group with MCT placebo supplement. Study assessments were performed on the first day 0 (D0, baseline) and day 90 (D90) on the last day of supplementation.

The study was conducted in accordance with Good Clinical Practices (Committee for Proprietary Medicinal Products, July 1996), the law of December 20, 1988 (n° 2004-806 of August 9, 2004) and the Helsinki Declaration (1964). Participants were informed about the nature, purpose, benefits, and risks of participation in this study, and they signed informed consent form before the start of the clinical study.

3.1- Participants

Caucasian women aged 25-60 years, with skin phototypes II and III were included in the study with inclusion and exclusion criteria. All skin types were included (dry skin, combination skin, oily skin) with SS verified by stinging test.

3.2- Clinical evaluation & instrumental measurements

3.2.1- Measurement of Transepidermal Water Loss (TEWL)

TEWL was measured with the vapometer® (Delfin Technologies, Kuopio, Finland).
Three measurements were taken using identical area on each visit (at the lobe of the right ear, at 4cm on a line connecting the lobe to the commissure and at the labial commissure). The result was the mean of 3 measurements. The TEWL mean value was expressed as the mass of water evaporated per unit area and time (g/m²/h).

3.2.2-Dermatological scoring of cutaneous redness

The 2D photographs of the entire face were taken with the LifVizMini camera. 3D photographs were used with the Quantificare LifVizApp software to enhance the visibility of cutaneous microcirculation and redness in people with SS. Using these photographs allows for better visualization of improvements in skin sensitivity after treatment with the dietary supplement. A redness intensity score scale from 0 to 4 was used: None (0), Slight (1), Moderate (2), Significant (3), Severe (4).

4- Statistical analysis

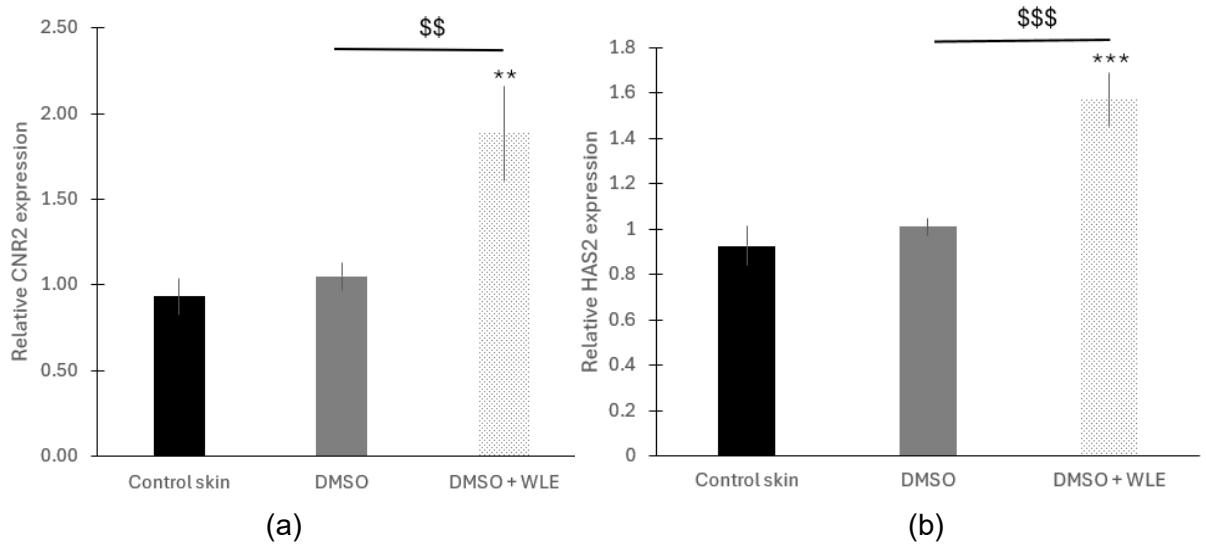
For transcriptomic and protein analysis, Welch's T-test was employed to assess differences between groups. This test was selected due to its ability to account for unequal variances and sample sizes.
For non-clinical part, mean and standard deviation were calculated. After checking the normality of the groups by Shapiro-Wilk test, the comparison of the parameter was carried out with the paired t test or Wilcoxon test (alpha risk of 5%) from PRISM10.
For clinical data, comparisons were drawn over time (D0 versus D90) and between products. For intragroup comparison, a two-tailed Student's statistical T-test for paired data was performed with an alpha risk of 5% if the data collected followed a normal distribution (verified by the Shapiro Wilk test). Otherwise, a non-parametric Wilcoxon test was performed with an alpha risk of 5%. In the same way, a two-tailed Student's statistical test for unpaired data was performed with an alpha risk of 5% or a non-parametric Mann-Whitney test was performed with an alpha risk of 5% for intergroup comparison.

3. Results

1- From Gene Expression to Protein: A Comprehensive Analysis

1.1-WLE treatment stimulated the expression of CNR2 and HAS2 mRNA in skin explants

As part of a comprehensive study, CNR2 and HAS2 gene expressions have been examined to explore their regulation after WLE treatment. Among the findings, Figure 1 illustrates that WLE treatment significantly upregulated CNR2 of 88% - a key receptor in the endocannabinoid system – and HAS2 of 50% – the Hyaluronan Synthase 2 enzyme. These results represent a subset of the broader dataset analyzed.

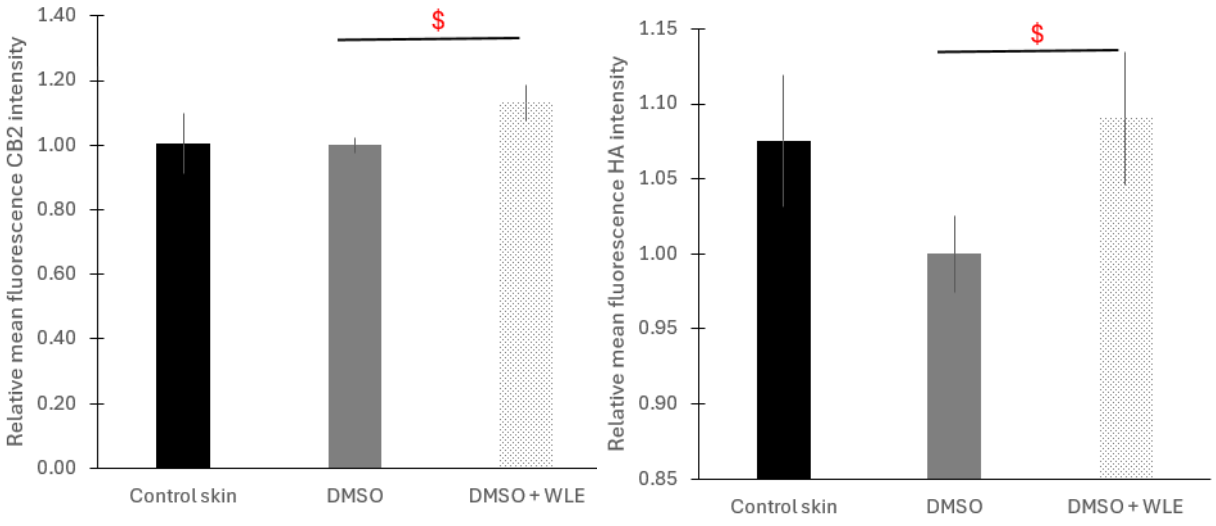


(a) (b)

Figure 1 : Influence of WLE treatment at 6,7µg/mL on (a) CNR2 and (b) HAS2 gene expression. Results are expressed as relative mean expression ± SEM. Statistical analysis was performed using a two-tailed unpaired Student's T-test ** p<0.01, *** p<0.001 compared with the skin control, $$p<0.01, $$$ p<0.001 compared with DMSO.

1.2- WLE induces the expression of CB2 protein and HA in skin explants.

In Figure 2, the results suggest a noticeable trend of increased CB2 and HA fluorescence intensity following WLE treatment, indicating a potential increase in CB2 and HA protein levels in the skin.



(a) (b)

Figure 2 : Influence of WLE treatment at 6,7µg/mL on (a) CB2 and (b) Hyaluronan (HA) protein expression. Results are expressed as relative mean fluorescence intensity ± SEM. Statistical analysis was performed using a two-tailed unpaired Student's T-test $ p<0.1 compared with DMSO.

1.3-WLE treatment reduced TRPV1 expression on sensitive skin model

As presented in Figure 3, TRPV1 receptor expression was significantly induced (4.5-fold) in ISS model in comparison to control skin. After treatment with WLE, TRPV1 receptor expression decreased significantly by 26.3% compared to the ISS condition.

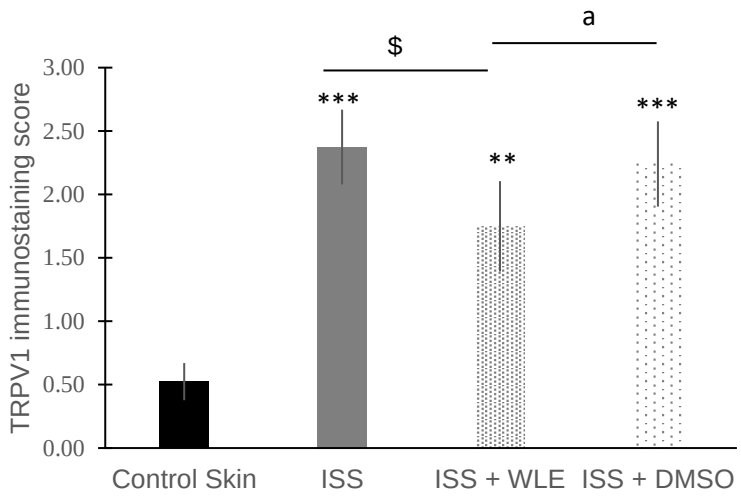


Figure 3: Influence of WLE on TRPV1 expression after induction of skin sensitivity. Immunohistochemical evaluation of TRPV1 expression in epidermis (approximately 15 fields at x 400 magnification). Results are expressed in immunostaining score as mean ± SEM. *** p<0.001, ** p<0.01 compared with control skin, $ p<0.05 compared with ISS, a p<0.05 compared with ISS + DMSO.

1.4-WLE treatment increased Mast cells granulation on sensitive skin model

As shown in Figure 4, the percentage of mast cells with no or light granulation (score 1) was significantly increased in the ISS model (3.5-fold) compared to control skin. Following treatment with WLE, the percentage of mast cells with score 1 decreased significantly by 61.6% compared to the ISS model.

Logically, the percentage of mast cells with important granulation (score 3) was significantly decreased in the ISS model by 56% compared to control skin. After treatment with WLE, the percentage of mast cells with score 3 increased significantly (2-fold) compared to the ISS model.

The percentage of mast cells with moderate granulation (score 2) was not significantly altered in the ISS model compared to control skin. Neither WLE nor DMSO treatment modified the percentage of mast cells with score 2 compared to the ISS model.

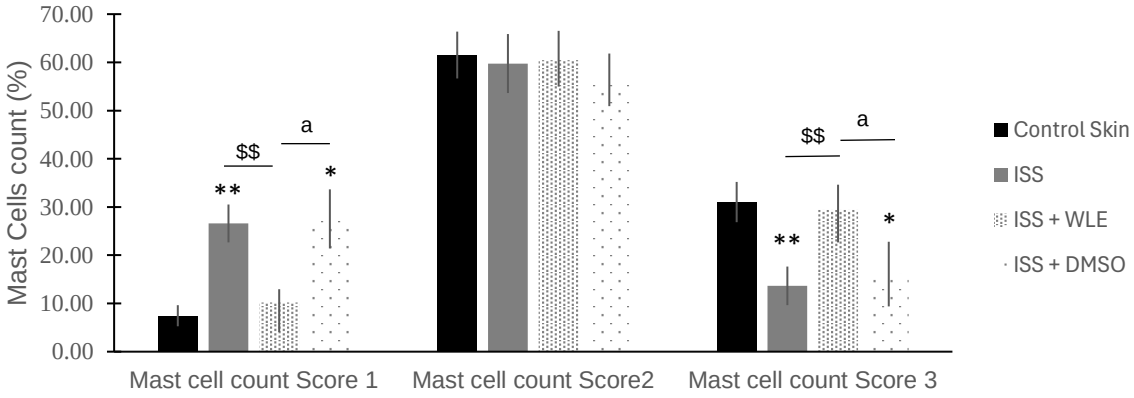
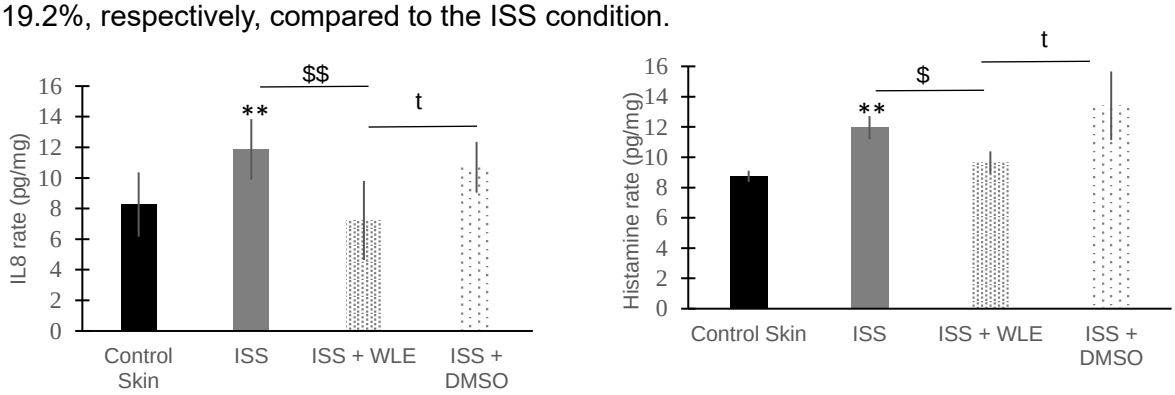


Figure 4: Influence of WLE on mast cells score after induction of skin sensitivity. Mast cells were stained with toluidine blue. Approximately 15 fields at x 400 magnification were analyzed. Mast cells were quantified to establish percentage related to the different scores: * p<0.05, ** p<0.01, compared with control skin explants, $$ p<0.01 compared with ISS; a p<0.05 compared with ISS + DMSO.

1.5-WLE treatment reduced IL8 and histamine on sensitive skin model

As shown in Figure 5, IL8 and histamine levels were significantly increased in the ISS model by 43.5% and 36.7%, respectively, compared to control skin.

After treatment with WLE, IL8 and histamine levels decreased significantly by 39.1% and 19.2%, respectively, compared to the ISS condition.



(**a**) (**b**)

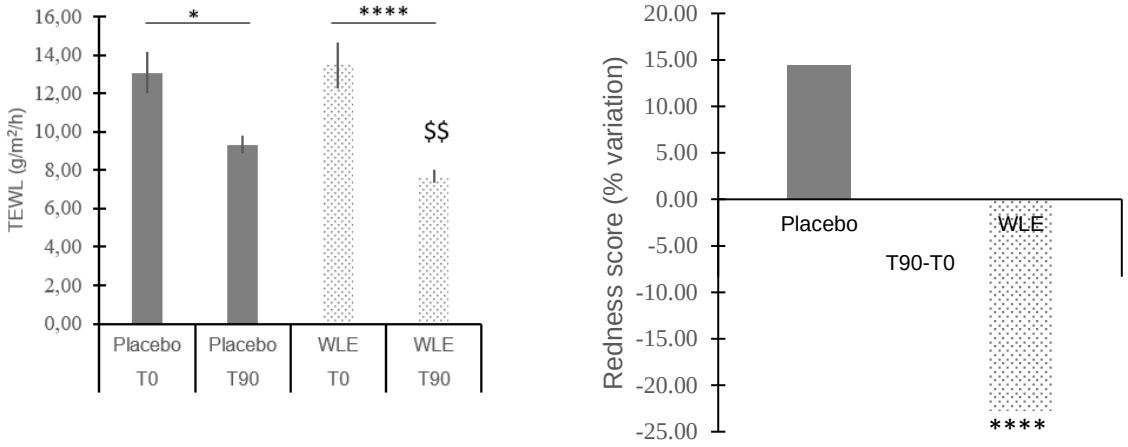
Figure 5. Influence of WLE on a) IL8 and b) histamine rates after induction of skin sensitivity. IL8 and histamine rates were quantified after ELISA assay on culture supernatants. Results are expressed in pg/mg as mean ± SEM. ** p<0.01 compared with control skin explants, $ p<0.05, $$ p<0.01 compared with ISS, t p<0.1 compared with ISS + DMSO.

2-Clinical Study

The clinical study was conducted on 60 women with SS and a mean age of 50.1 ± 10.6 years. The repartition regarding skin type was 2% of subjects with oily skin, 7% with normal skin, 36% with dry skin and 55% with mixed skin. Two volunteers from WLE group had to be withdrawn from the study for medical reasons unrelated to dietary supplement intake.

2.1-WLE supplementation decreased TEWL and dermatological scoring of Cutaneous redness

As shown in figure 6, a significant decrease of 28.6% in TEWL in the placebo group after 90 days was observed while after 90 days of WLE supplementation, TEWL decreased significantly of 43.0%. Thus after 90 days of supplementation, the TEWL was significantly reduced by 17.7% in the WLE group compared to the placebo group (p=0.00662). Regarding the redness score a significant increase of 14.4% in cutaneous redness were observed after 90 days in the placebo group while after 90 days of WLE supplementation cutaneous redness was reduced by 22.7%.



(**a**) (**b**)

Figure 6 (a) Influence of WLE supplementation on TEWL results are expressed in g/m^2/h as mean ± SEM. * p<0.05 compared with placebo T0, **** p<0.0001 compared with WLE T0, $$ p<0.01 compared with placebo T90. (b): Influence of WLE supplementation on redness score variation. Results are expressed in redness score variation in %. **** p<0.0001 compared with placebo.

4. Discussion

Modern advancements have revolutionized dermo-cosmetic research through human *ex vivo* skin biopsies. These models, closely mimicking living skin, enable precise evaluation of active ingredients prior to clinical trials, improving understanding of their efficacy and safety.

Sensitive skin is closely linked to inflammation due to the hyperactivity of sensory receptors like TRPV1, which amplify irritation in response to external triggers. This leads to the release of pro-inflammatory signals, such as IL8, histamine, and the degranulation of macrophages, further weakening the skin barrier and exacerbating redness, discomfort, and irritation. [3] [4] In this study, *ex vivo* models confirmed that TRPV1 was activated in sensitive skin, along with an increase in IL8, histamine, and macrophage activity. However, treatment with WLE significantly reduced these inflammatory markers, showcasing its anti-inflammatory properties. Moreover, WLE treatment resulted in an 88% increase in CNR2 expression, which plays a crucial role in immunomodulation and pain relief [5] [6] [7]. This result is consistent with findings from protein analysis associated with CB2. These results highlight WLE's potential to improve anti-inflammatory effects and skin health in individuals with sensitive skin.

Building on the *ex vivo* findings, which demonstrated promising efficacy of WLE, a clinical study was set up to assess whether these results could be replicated and confirmed in a human food supplementation context. Clinical studies play a crucial role in scientific research by validating the efficacy and safety of treatments directly in human subjects. This approach ensures a thorough understanding of outcomes, fostering innovation and advancing evidence-based solutions in fields like dermatology and nutricosmetics.

Clinical results demonstrated that a decrease in TEWL is directly linked to an improvement in the integrity of the skin barrier function [8]. Sensitive skin, often characterized by a weakened barrier, benefits significantly from WLE, which has been shown to strengthen this protective layer. Additionally, sensitive skin is prone to issues such as redness and discomfort,

which are also alleviated by WLE treatment. These findings align cohesively with previous data across various studies, reinforcing WLE's role in enhancing the skin's resilience and addressing key challenges associated with sensitive skin.

On another note, WLE demonstrated its ability to increase hyaluronan (HA) levels, as shown by a 9% increase at a concentration of 6.7 µg/mL in this study. This effect, in line with genomic analyses, highlights its potential to improve extracellular matrix (ECM) repair and maintenance, enhance the wound healing process, and promote skin hydration [9] [10] [11]. These findings emphasize the interest of WLE in supporting skin health.

5. Conclusion

Through this R&D program, the mechanism of action of WLE on skin inflammation regulation has been studied and its efficacy in relieving skin sensitivity when taken orally has been demonstrated. These results allow for the consideration of innovative formulations of WLE in nutricosmetic and dermatological applications.

References

[1] Farage, “The Prevalence of Sensitive Skin,” *Front Med,* p. 6:98, 2019 May.

[2] Misery, “Peaux sensibles, peaux réactives [Sensitive skin, reactive skin],” *Ann Dermatol Venereol,* Vols. 146(8-9), pp. 585-591, 2019.

[3] F. M. M. H. Berardesca E, “Sensitive skin: an overview,” *Int J Cosmet Sci,* vol. 35, no. 1, pp. 2-8, 2013.

[4] P. A. Inamadar AC, “Sensitive skin: an overview.,” *Indian J Dermatol Venereol Leprol,* vol. 79, no. 1, pp. 9-16, 2013.

[5] R. S. S. P. S. O. K. S. Anthony AT, “Cannabinoid Receptors and Their Relationship With Chronic Pain: A Narrative Review.,” *Cureus,* p. 12(9), 2020.

[6] P. A. C. C. V.-C. M. Correia-Sá I, “ Cutaneous endocannabinoid system: Does it have a role on skin wound healing bearing fibrosis?,” *Pharmacol Res.,* p. 159:104862. , 2020.

[7] W. L. L. M. J. S. Z. M. T. Z. W. M. L. J. Z. R. G. D. Li SS, “Cannabinoid CB_2 receptors are involved in the regulation of fibrogenesis during skin wound repair in mice.,” *Mol Med Rep.,* vol. 13(4), pp. 3441-50., 2016.

[8] E. P. F. K. Grubauer G, “Transepidermal water loss: the signal for recovery of barrier structure and function,” *J Lipid Res,* vol. 30(3), pp. 323-33, 1989.

[9] Š. R. P. V. Š. M. Š. B. H. M. N. K. V. V. Žádníková P, “The Degradation of Hyaluronan in the Skin.,” *Biomolecules,* vol. 12(2):, p. 251., 2022.

[10] L. d. R. C. P. Y. Evrard C, “Epidermal Hyaluronan in Barrier Alteration-Related Disease,” *Cells,* vol. 10(11), p. 3096, 2021.

[11] R. M. K. G. Papakonstantinou E, “Hyaluronic acid: A key molecule in skin aging,” *Dermatoendocrinol.,* vol. 4(3), pp. 253-8., 2012.