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An innovative ingredients combination to promote skin longevity that revitalizes and protects mitochondrial function and addresses problems of aged and impaired skin.

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1. Introduction

Mitochondria serve as the bioenergetic center of eukaryotic cells, acting as central regulators of cellular homeostasis and primary targets in both chronological and UV-induced skin aging[1]. A comprehensive review has identified 14 hallmarks of aging including mitochondrial dysfunction[2]. Mitochondria functionally link to multiple aging-related pathways[3]. With aging, mitochondrial function deteriorates, which compromises the contribution of mitochondria to cellular bioenergetics, enhances the production of reactive oxygen species (ROS), and may trigger inflammation and cell death[4]. The deterioration of mitochondrial function leads to aging phenotypes.

Mitochondria not only generate cellular energy (ATP) to sustain physiological functions but also produce metabolic byproducts such as mitochondrial ROS (mtROS).The Free Radical Theory of Aging, proposed by Harman in the 1950s, posits that mitochondrial damage and dysfunction—primarily driven by dysregulated ROS metabolism—constitute the initiating events of aging[5, 6]. Accumulating evidence confirms that mitochondrial dysfunction and ROS-induced oxidative stress are defining features of aged tissues, including skin, and are directly associated with cutaneous aging phenotypes such as wrinkles, graying hair, alopecia, uneven pigmentation, and delayed wound healing[7, 8]. Excessive ROS can promote the expression of pro-inflammatory cytokines by activating transcription factors such as NF-κB, and pro-inflammatory cytokines can induce cellular aging. Reducing ROS is of great significance for intervening in inflammation and delaying aging[9].

Mitochondria-targeted skincare strategies aim to restore bioenergetic capacity ("engine revitalization") and mitigate mtROS generation ("exhaust reduction") at their source. By addressing mitochondrial dysfunction, such interventions may rejuvenate skin vitality, offering a mechanistic approach to counteract aging-related decline of skin function.

In this study, we built a platform to conduct systematic research on mitochondrial functions and obtained a combination of *galactomyces* ferment filtrate, *saccharomyces* polypeptides and polyamino sugar condensate. *In vitro* tests had confirmed that the combination endogenously activates and protects various types of skin cells. *In vivo* tests had confirmed that the combination improved skin brightness, reduced yellowness and redness, repaired skin barrier, moisturized and tightened skin.

2. Materials and Methods

Cell culture

Human immortified epidermal cells (HaCaT), human skin fibroblasts (HFF-1), mouse mononuclear macrophages (RAW264.7) were resuscitated. When the plate spreading rate reached about 60%, cells were inoculated into 96-well plates and incubated overnight in a CO_2 incubator (37 °C, 5% CO_2). Cells were then divided into normal control group (NC), model control group (MC), and sample group (with specified concentration). When cell paving rate reached about 50%, the NC and the MC were added fresh culture medium, and samples of a specific concentration were added to the MC. Then cells were incubated in a CO_2 incubator (37 °C, 5% CO_2) for 24 h. Stimulus modeling (UVA, UVB, LPS, H_2O_2) was carried out and cells were incubated overnight. After incubation, culture medium was removed and detection was conducted.

Zebrafish test

Transgenic neutrophil green fluorescent zebrafish (MPX) at two days after fertilization were used. Then a SLS stimulus modeling or an inflammatory model of caudal fin injury in zebrafish were carried out. They were divided into NC, MC, and sample group (with the specified concentration). After incubating in the dark at 28 °C, number of neutrophils in the skin or caudal fin region of zebrafish was analyzed using a fluorescence microscope.

Reagents

Enhanced ATP assay kit and NAD^+/NADH assay kit were purchased from Beyotime. PrestoBlue™ cell viability reagent was purchased from Invitrogen. MitoTracker™ Green FM, IL-1β and TNF-α ELISA kits, and MitoSOX™ Red mitochondrial superoxide indicator were purchased from Thermo Fisher. Mitochondrial Membrane Potential Assay Kit with JC-1 was from Solarbio. Microtubule-Associated Protein 1 Light Chain 3b (LC3b) antibody was from Abcam.

Gene expression analysis

Following experimental treatments, culture medium was removed, and total RNA was extracted from each experimental group. cDNA was synthesized and subjected to quantitative real-time PCR (qRT-PCR) to evaluate the expression levels of target genes. In UVB-irradiated HaCaT cells, the expression level of PGC-1α and FOXO1 was analyzed. The expression level of SIRT3 was analyzed under the condition of UVA stimulation.

In Vivo Evaluation

32 subjects aged 18-60 with sensitive skin applied 2% combination-containing serum twice daily for 4 consecutive weeks. Self-assessed scoring of lactic acid stinging tests was performed by participants. Skin parameters were evaluated using the VISIA-CR system to quantify the following metrics: crow's feet area ratio, a* values (redness), b* values (yellowness), and Individual Typology Angle (ITA). Transepidermal water loss (TEWL) and stratum corneum hydration levels were measured using the Cortex Dermalab Combo device.

Statistical analysis

Data were presented as mean±standard deviation. Statistical analyses were performed by GraphPad Prism, using Student's t-test (paired, two-tailed) or multiple comparisons (one-way, two-way ANOVA) based on the experimental data. Differences with a value of $p<0.05$ were considered statistically significant.

3. Results

3.1 Cellular and mitochondrial energy

Cell viability is an indicator of the proportion of healthy, viable cells within a population[10]. NAD^+ and NADH are essential coenzymes in cellular metabolism. The NAD^+/NADH ratio affects the cell's ability to generate energy. Mitochondrial membrane potential (ΔΨm) serves as a critical determinant of mitochondrial structural/functional integrity. To evaluate the effects of combination on cellular and mitochondrial energy, experiments on cell viability, NAD^+/NADH ratio and mitochondrial membrane potential (ΔΨm) were conducted as outlined in Figure 1. The combination significantly enhanced cell viability (49%), ATP levels (33%) and NAD^+/NADH ratio (39%) as HaCaT cells were stimulated by UVB. ΔΨm was increased by 32%, as HFF-1 cells were stimulated by UVA. The results demonstrated our combination's capacity to augment cellular and mitochondrial energetic functions.

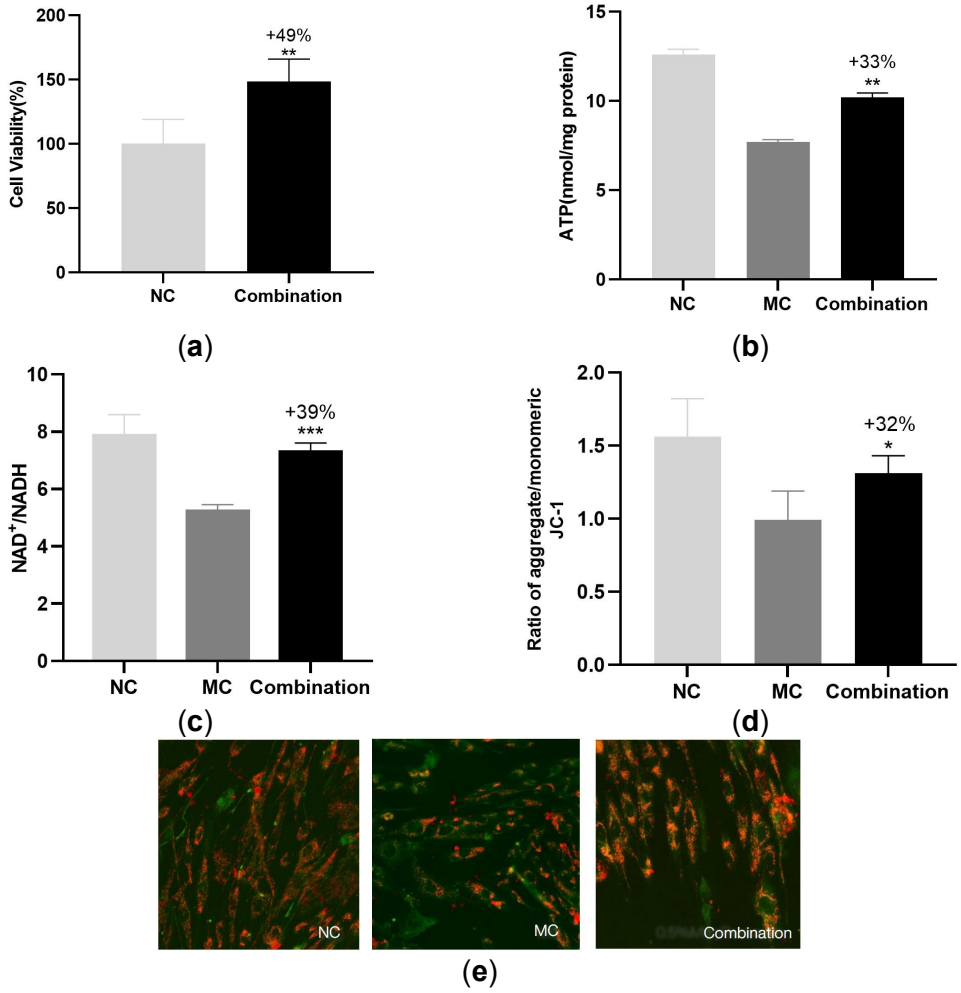


Figure 1. *In vitro* tests of cellular and mitochondrial energy. (a) Cell viability; (b) ATP content; (c)NAD^+/NADH; (d) Mitochondrial membrane potential (ΔΨm); (e) JC-1 fluorescence contrast of ΔΨm. *P<0.05,**P<0.01, ***P<0.001,****P<0.0001.

3.2 Mitochondrial quality control

To assess the mitochondrial protective efficacy of the combination, key functional parameters of the mitochondrial quality control were analyzed. PGC-1 α is a master regulator of mitochondrial biogenesis[11]. As demonstrated in Figure 2.a-d, the combination significantly increased mitochondrial mass (59%), networks (67%), distribution coverage (61%). Concurrently, it upregulated PGC-1 α gene expression (44%), indicating enhanced mitochondrial biogenesis (Figure 2.e). These findings collectively demonstrated that the combination improved mitochondrial dysfunction and sustained physiological homeostasis.

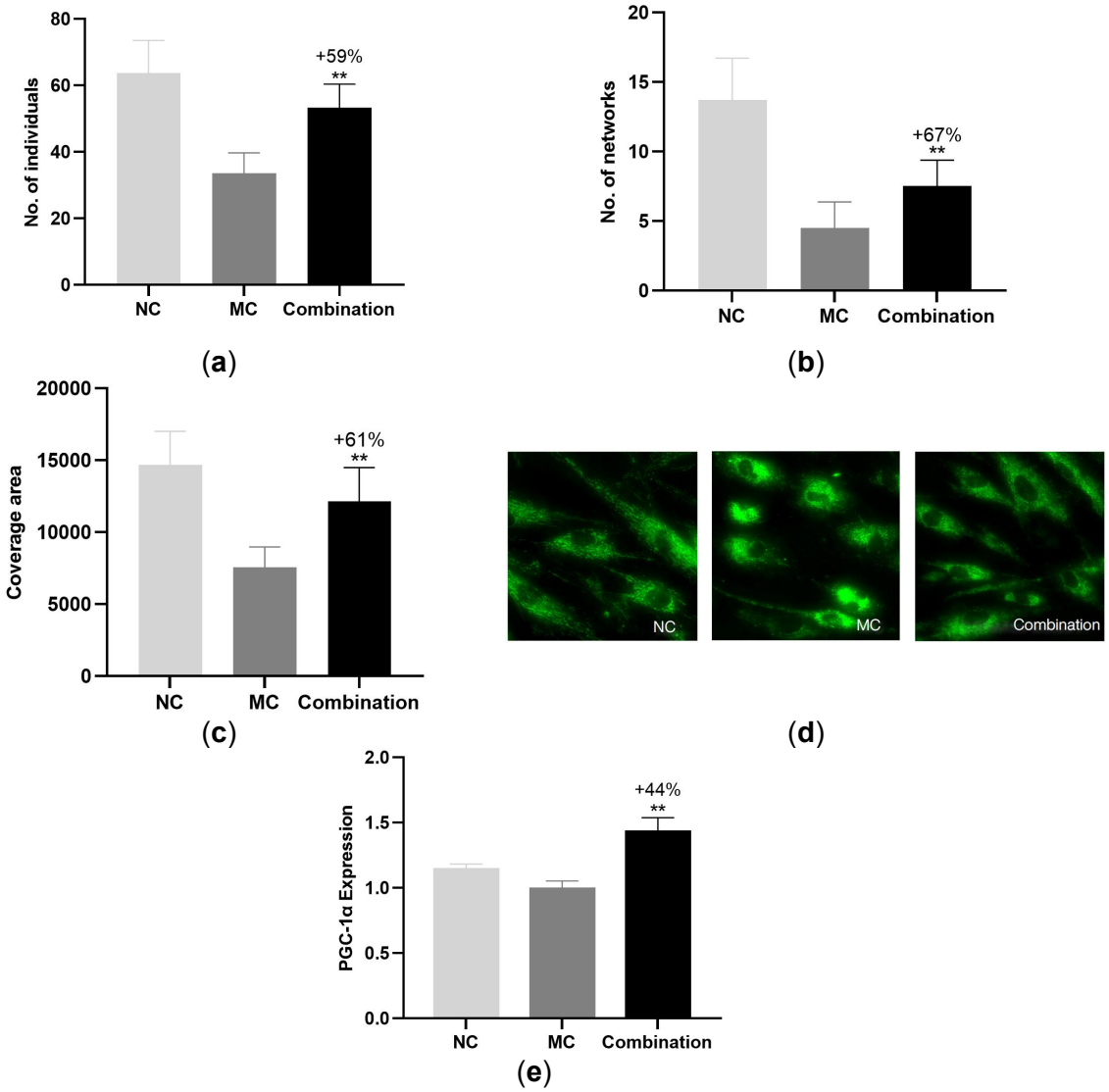


Figure 2. *In vitro* tests of mitochondrial quality control. (a) Number of mitochondria; (b) Mitochondrial network; (c) Mitochondrial coverage area; (d) Contrast fluorescence of mitochondrial coverage area; (e) PGC-1α gene expression. *P<0.05,**P<0.01, ***P<0.001,****P<0.0001.

3.3 Longevity-associated protein SIRT3

Longevity-associated proteins such as SIRT3, a mitochondrial deacetylase, regulate oxidative stress responses and mitochondrial energy metabolism. As fibroblast cells were stimulated by UVA and treated by the combination，the gene expression of SIRT3 increased by 44%. This result suggested that UV radiation leaded to a decrease in the expression of SIRT3, and our combination could effectively protect SIRT3 (Figure 3).

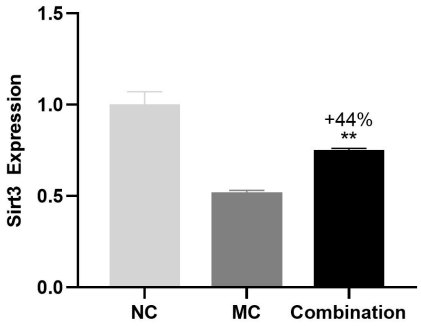


Figure 3. *In vitro* tests of SIRT3 gene expression. *P<0.05,**P<0.01, ***P<0.001,****P<0.0001.

3.4 Autophagy and mitophagy

Autophagy and mitophagy are ways for cells to maintain health by breaking down and eliminating damaged or unnecessary cellular components. The coordination of autophagy and mitophagy (via ATG7 and LC3 activation) directly influence cellular senescence and redox balance. Experiments were performed as depicted in Figure 4. Compared to MC group, the combination significantly upregulated LC3 expression by 45% (Figure 4.a), ATG7 expression by 43% (Figure 4.b), critical for autophagosome formation pivotal for mitochondrial redox regulation. Furthermore, compared to NC group, the combination group exhibited a 21% increase in LC3B/mitochondria colocalization intensity (Figure 4.c-d), a key marker of mitophagic flux. The stronger co-localization signal of intensity of autophagy occurrence in mitochondria, reflected the higher level of mitochondrial autophagy. These results confirmed robust activation effect of cellular and mitochondrial clearance mechanisms of the combination.

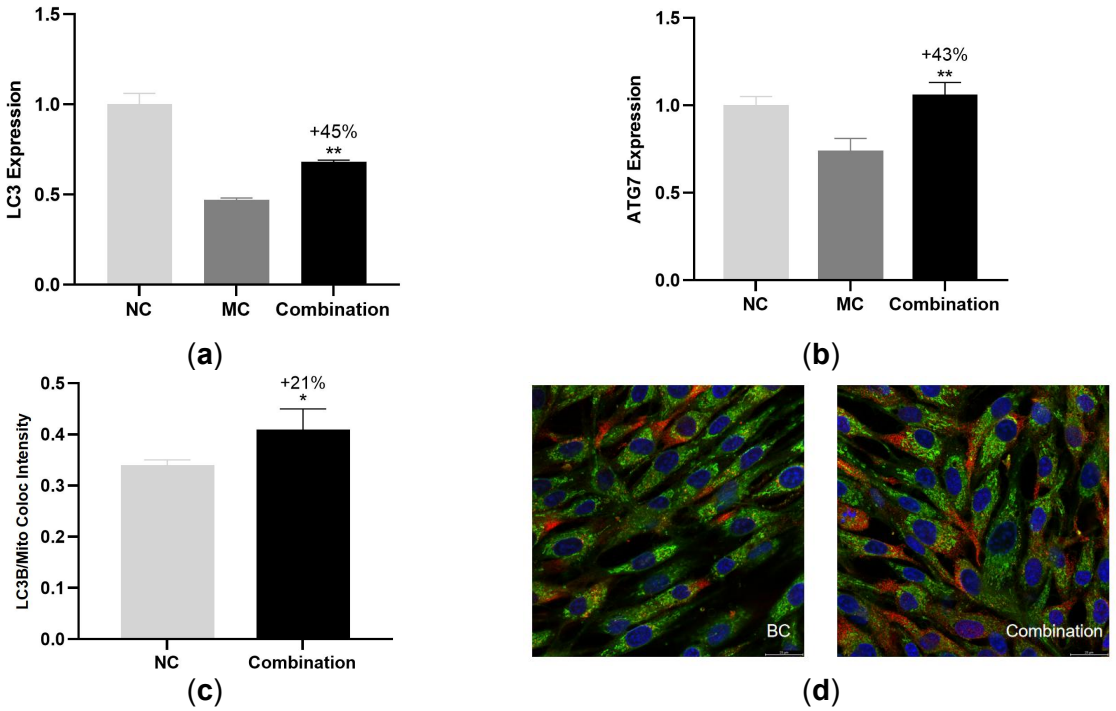


Figure 4. *In vitro* tests of mitochondrial autophagy and longevity proteins. (a) LC3B gene expression; (b) ATG7 gene expression; (c) LC3B colocalized with mitochondria; (d)

Fluorescence image of LC3B colocalized with mitochondria; Blue fluorescence represented the cell nucleus, green fluorescence indicated the morphology of mitochondria, and red fluorescence showed LC3B. *P<0.05,**P<0.01, ***P<0.001,****P<0.0001.

3.5 Anti-oxidative stress

Oxidative stress, driven by mtROS overproduction and compromised antioxidant defenses, initiates metabolic dysregulation[12]. Our findings demonstrated that the combination significantly reduced mtROS accumulation (27%, Figure 5.a) while activating FOXO1-mediated antioxidant pathways (31%, Figure 5.b) – a master transcriptional regulator of cellular redox homeostasis and stress adaptation[13]. By preserving electron transport chain integrity and preventing ROS-induced biomolecular damage, the combination enhanced cellular capacity to neutralize ROS. The combination breaked the vicious cycle of oxidative damage and prevented further overproduction of ROS, thereby slowing down macromolecular damage. Through this antioxidant mechanism, the combination reduced skin aging markers, pigmentation, and structural tissue deterioration by restoring redox balance.

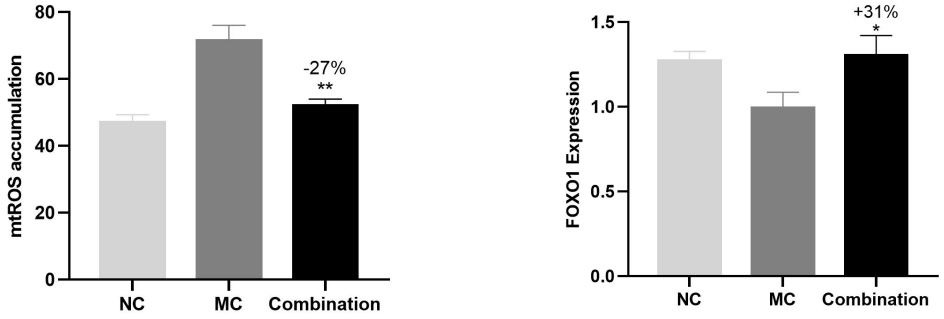
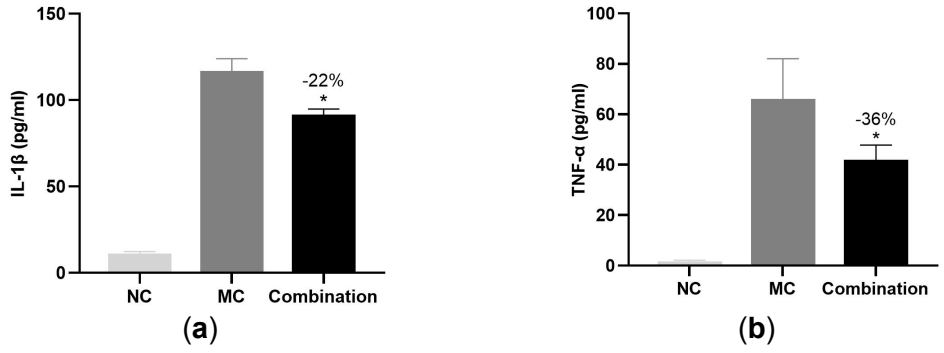


Figure 5. *In vitro* tests of anti-oxidative stress capacity. (a) mtROS accumulation; (b) FOXO1 gene expression. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

3.6 Against inflammatory damage

Integrative analysis of multi-model datasets (Figure 6) showed that the combination achieved comprehensive anti-inflammatory and tissue repair effects by synergistically regulating the inflammatory cascade mediated by oxidative stress. In LPS-stimulated cell experiments, the combination significantly inhibited the secretion of proinflammatory cytokines, with IL-1 β reduced by 22% and TNF-α reduced by 36% compared with the control group (Figure 6.a-b); while in the zebrafish injury model, the combination showed a 30% soothing effect and a 46% repair effect (Figure 6.c-d). Our results confirmed the dual mechanistic effects of the combination: alleviating inflammatory overactivation by blocking oxidative stress pathways and activating endogenous repair programs.



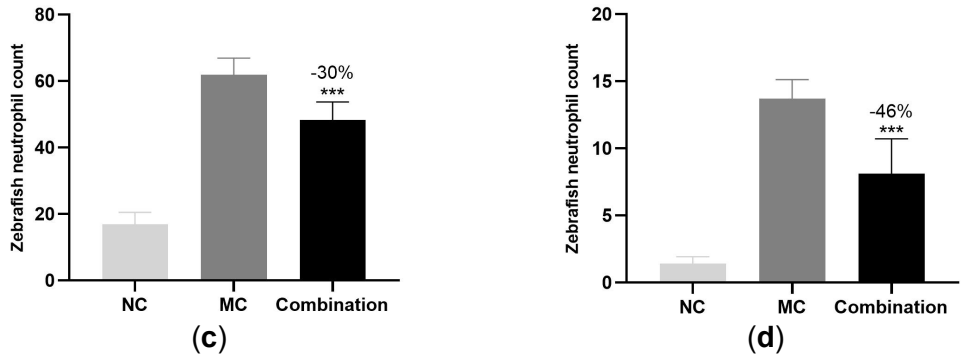
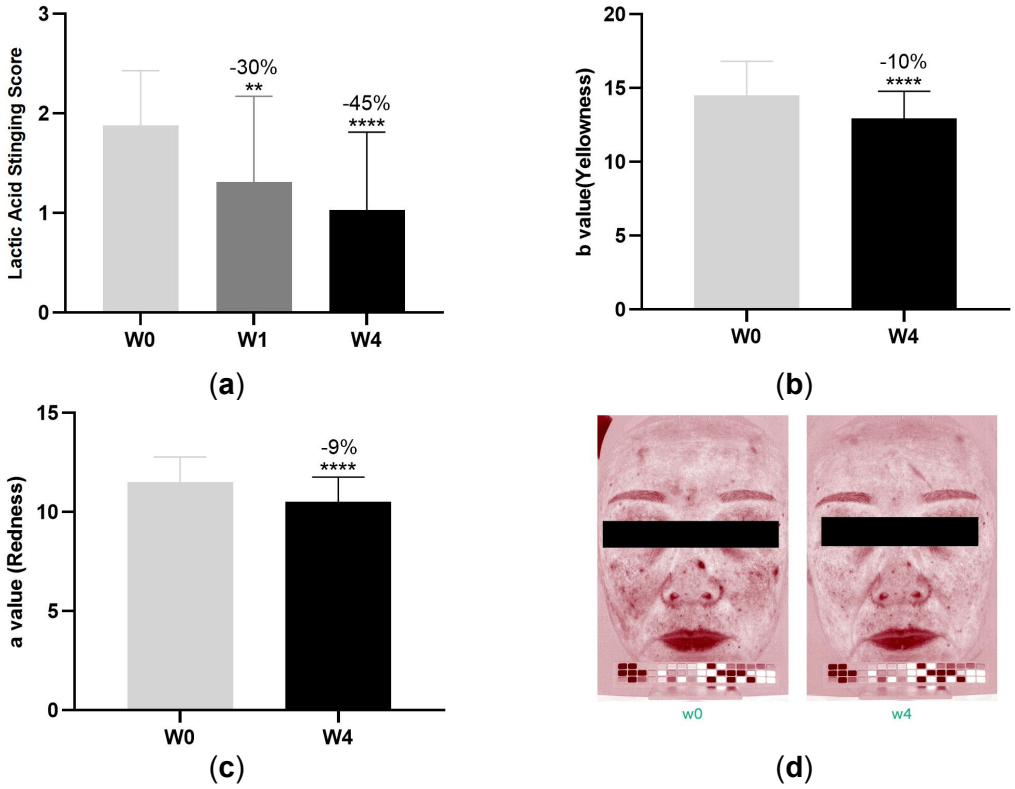


Figure 6. *In vitro* tests of anti-inflammatory, soothing and repairing. (a) IL-1β; (b) TNF-α; (c) Zebrafish soothing efficacy test; (d) Zebrafish repair efficacy test. *P<0.05,**P<0.01, ***P<0.001,****P<0.0001.

3.7 Clinical efficacy trial

A clinical study involving 32 sensitive skin subjects demonstrated the combination's efficacy. The combination significantly reduced neurosensory irritation (Figure 7.a) while improving skin tone parameters through decreased a* values (redness) and b* values (yellowness) alongside elevated ITA (Figure 7.b-e). Barrier restoration was evidenced by 26% reduction in TEWL and 14% hydration increase (Figure 7.f-g), confirming its dual repair-moisturization capacity. Notably, mitochondrial functional improvements translated to anti-aging outcomes: a 19% reduction in crow's feet area ratio after 4 weeks (Figure 7.h-i), reflecting enhanced dermal structural integrity. These results collectively positioned the combination as a mitochondrial-modulating therapy addressing reactive skin dyschromia, barrier compromise, and photoaging manifestations in a synchronized manner.



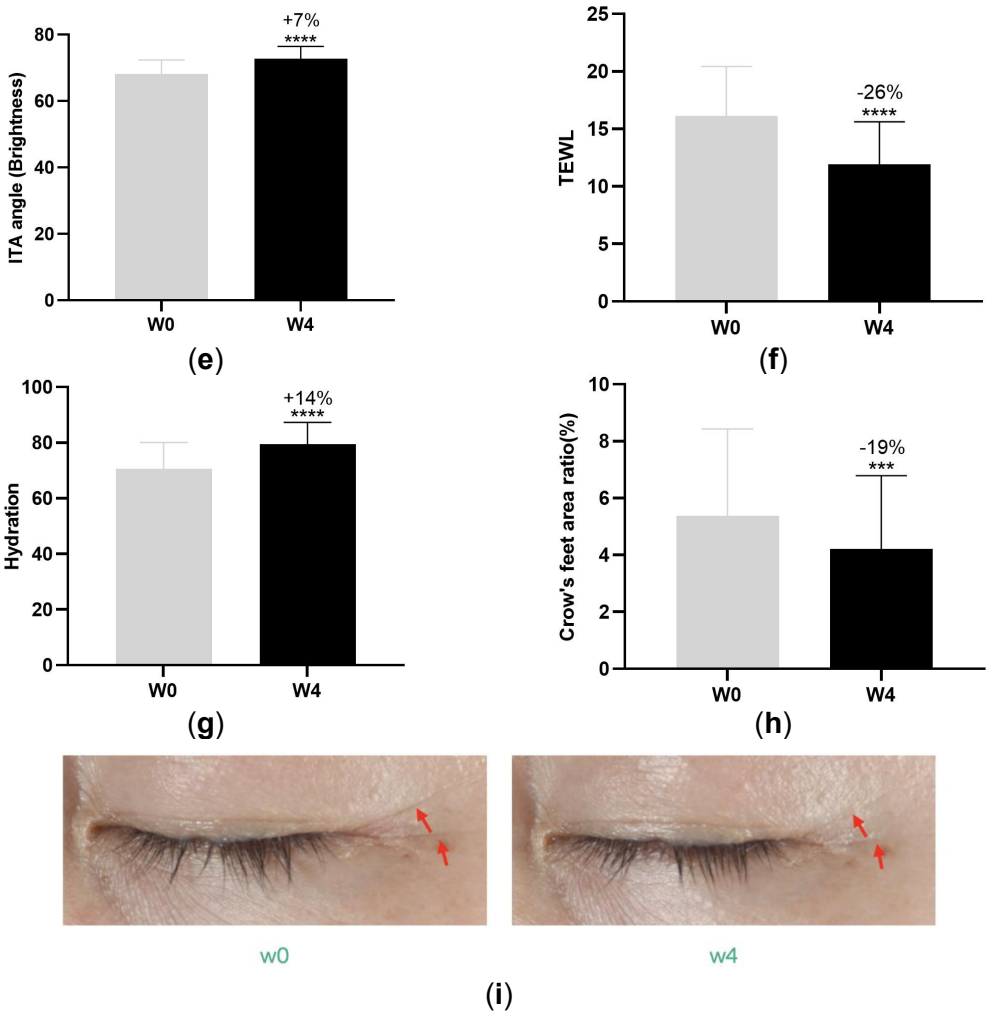


Figure 7. *In vivo* tests. (a) Lactic acid stinging evaluation; (b) b* value (yellowness); (c-d) a* value (redness); (e) Skin brightness; (f) Transepidermal water loss; (g) Stratum corneum water content; (h-i) Crow's feet area ratio. *P<0.05,**P<0.01, ***P<0.001,****P<0.0001.

4. Discussion

This study elucidated the multi-targeted mechanism of action of a novel mitochondria-targeted skincare combination of *galactomyces* ferment filtrate, *saccharomyces* polypeptides and polyamino sugar condensate, through systematic *in vitro* and *in vivo* validation. The combination modulated cellular energy metabolism to counteract skin aging, photoaging, and inflammatory responses. Its pleiotropic effected simultaneously target intrinsic aging pathways (e.g., mtDNA repair) and extrinsic damage cascades (e.g., oxidative stress), with demonstrated efficacy across UVB/UVA irradiation and H_2O_2-induced oxidative stress models.

Mitochondria play a central role in cellular energy metabolism, signal transduction, and biosynthetic processes. We tested the protective effect of our composition on mitochondrial function. Mitochondrial membrane potential and mitochondrial morphology and quality control directly regulate mitochondrial architecture, homeostasis, and functional capacity. As a master regulator of mitochondrial biogenesis, PGC-1α is essential for preserving mitochondrial quantity and functional competence[11]. The result suggested that the combination effectively improved these indicators and played an important role on mitochondrial protection.

Mitochondria also play a pivotal role in aging and inflammatory pathologies, acting as both regulators and targets. Age-related mitochondrial dysfunction includes elevated ROS production[14]. In senescent cells, dysfunctional mitochondria generate excess ROS, which stabilizes the senescence-associated secretory phenotype (SASP) and drives neighboring cell senescence[15]. When senescent cells accumulate in tissues, they became "zombie cells" and secrete inflammatory factors[16, 17]. The alterations impair energy homeostasis and activate stress-response pathways, including the NLRP3 inflammasome, which links mitochondrial damage to chronic inflammation[18]. For instance, ROS-mediated mtDNA leakage into the cytosol triggers cGAS-STING signaling, amplifying pro-inflammatory cytokine release (IL-1 β , TNF- α)[19], impairing barrier function, and promoting hyperpigmentation[20]. Our combination suppressed mtROS (27%), senescence-associated pro-inflammatory cytokines IL-1β (22%) and TNF-α (36%), demonstrating its function on inhibit inflammatory aging. Additionally, enhancing mitophagy has shown promise in reversing age-related mitochondrial accumulation. And our combination could effectively improved mitophagy (LC3 and ATG7) and SIRT3 (44%).

5. Conclusion

In this study, we built a platform to conduct systematic research on mitochondrial functions and obtained a combination of *galactomyces* ferment filtrate, *saccharomyces* polypeptides and polyamino sugar condensate. Systematic *in vitro* and *in vivo* validation demonstrated that the combination significantly enhanced cellular energy metabolism and mitochondrial function while exhibiting potent anti-inflammatory, antioxidant, and skin-rejuvenating effects. Key findings included a 33% increase in ATP production, 39% elevation in NAD^+/NADH ratio, and 49% improvement in cell viability, confirming robust metabolic activation. Mitochondrial restoration was evidenced by a 32% recovery of membrane potential and 44% upregulation of PGC-1α, coupled with a 27% reduction in mitochondrial ROS. Anti-inflammatory activity was highlighted by 22% and 36% decreases in IL-1β and TNF-α, while in the zebrafish injury model, the combination showed a 30% soothing effect and a 46% repair effect. Besides, autophagy activation was marked by 45% and 43% increases in LC3 and ATG7 expression. Skin benefits included 26% reduction in transepidermal water loss, 14% hydration boost, 19% decrease in crow’s feet area, and 7% improvement in skin brightness. The combination goes deep into the vital energy sources of skin cellular engines, endogenously empowers skin revitalization and protection, and directly addresses problems of aged and impaired skin.

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