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"Oxyresveratrol: A Novel, Gentle and Effective Skin Whitening Ingredient for Sensitive Skin"

Jianhua Zhang [1,2,*], Shichao Liu [1,2], Wenjiao Guo [1,2] and Yun Huang [1]

[1] N.O.D topia (GuangZhou) Biotechnology Co., Ltd. Guangdong Guangzhou, 510000, China; [2] Simpcare (GuangZhou) Biotechnology Co., Ltd. Guangdong Guangzhou, 510000, China.

1. Introduction

Melasma is a common acquired chronic hyperpigmentation disorder predominantly affecting sun-exposed areas, with prevalence varying from 1% to 50% across populations [1-4]. Epidemiological investigations have shown a higher prevalence of melasma in women and individuals with Fitzpatrick skin types II to IV [1]. The clinical symptoms are usually presented as centrofacial, malar, and mandibular skin patches [2], which can significantly impact patients' facial appearance and cause psychological distress, including anxiety disorder [5].

The etiology and pathogenesis of melasma are multifactorial, posing challenges for long-term management and increasing the risk of recurrence [4,6]. Key mechanisms include abnormal melanin production and distribution, increased solar elastosis, altered basement membrane, and vascular dilation [7]. Moreover, impaired barrier function has been observed in melasma lesional skin [8], suggesting that it may either play a role in the pathogenesis or be a concomitant feature of the condition. However, current therapies (e.g., hydroquinone) for melasma face limitations due to irritation risks, particularly in individuals with sensitive skin, with resulting suffering and condition aggravation for patients [3].

Sensitive skin is a syndrome defined by the occurrence of unpleasant sensations in response to stimuli that normally should not provoke such sensations [9]. Although its exact pathogenesis remains unclear, sensitive skin is widely believed to result from a combination of skin barrier dysfunction, sensory hyper-reactivity, and inflammatory or vascular hyperresponsiveness [10,11]. Furthermore, it affects 60-70% of adults globally [12], leading to both physical and psychological burdens on patients [13]. The high prevalence of sensitive skin underscored the importance of the mildness and tolerability of topical treatments.

Oxyresveratrol, a natural derivative of resveratrol, has drawn attention for its various activities, including antioxidant, anti-inflammatory, and anti-melanogenesis [14]. Previous studies have demonstrated that oxyresveratrol effectively inhibited tyrosinase activity and down-regulated the expression of microphthalmia-associated transcription factor (MITF), tyrosinase-related protein 1 (TRP-1), and dopachrome tautomerase (TRP-2) in mouse B16F10 melanoma cells

[15,16]. A clinical study of 60 female volunteers revealed that 0.2% oxyresveratrol significantly reduced melanin content in the upper arm after 4 weeks of application, and no adverse effects were observed [17], suggesting the safety and depigmenting effect of oxyresveratrol in clinics. However, the effects of oxyresveratrol on melasma treatment and the underlying mechanism were largely unknown.

This study aims to investigate the safety and efficacy of oxyresveratrol on melasma treatment and the underlying mechanism. Network pharmacology was used to screen compounds for treating hyperpigmentation and sensitive skin. The inhibitory effects of oxyresveratrol and three other resorcinol-based compounds on human tyrosinase were assessed *in vitro*. The impact of oxyresveratrol on melanogenesis and melanosome transport was examined using a co-culture system of B16F10 and HaCaT cells. A randomized, double-blind, placebo-controlled, 8-week clinical trial with 70 Chinese female participants was conducted to evaluate the efficacy and safety of 1% oxyresveratrol in treating melasma on sensitive skin. This study proved that oxyresveratrol could be safe and effective for the treatment of melasma through inhibiting both melanogenesis and melanosome transport.

2. Materials and Methods

2.1. Materials

Oxyresveratrol (CAS: 29700-22-9, purity ≥ 99%) was provided by Naturalis Srl (Italy). Resveratrol (CAS: 501-36-0, purity ≥ 98%) was obtained from Royal DSM (Netherlands). Phenylethyl resorcinol (CAS: 85-27-8, M866076, purity ≥ 98%) was purchased from MACKLIN (China). Glabridin (CAS: 59870-68-7, SG8480, purity ≥ 98%) was purchased from Solarbio (China). Recombinant human tyrosinase (TP321797L) was obtained from OriGene (USA). Reagents and kits for immunofluorescence were purchased from Servicebio (China). Other reagents were of the highest quality obtainable.

2.2. Network pharmacology analysis

The targets associated with sensitive skin and hyperpigmentation were collected from the human gene database, GeneCards. The targets associated with 15 skin-whitening compounds were collected from TCMSP, BATMAN, and SymMap database.

2.3. Human tyrosinase inhibition assay

The anti-tyrosinase activities of oxyresveratrol, resveratrol, phenylethyl resorcinol, and glabridin were assessed using the method of the previous study [18].

2.4. Cells and cell culture

The mouse B16F10 melanoma cells (CRL-6475) were obtained from ATCC (USA), and the human immortalized keratinocyte (HaCaT) cells were obtained from CLS (Germany). The cells were cultured in DMEM medium containing 10% FBS at 37℃ with 5% CO2. The B16F10 cells were seeded at a density of 1.5*10^4 cells in each 96-well plate and incubated for 24 h at 37℃, and then treated with different concentrations of oxyresveratrol in fresh medium and incubated for 24 h. The co-culture system of B16F10 and HaCaT cells was established using 6-well transwell plates as previously described [19].

2.5. Cell viability

The effect of oxyresveratrol on B16F10 cell viability was investigated by the CCK8 assay as previously described [20].

2.6. Measurement of melanin content

The cells were collected and dissolved in 1 mol/L NaOH containing 10% DMSO at 80℃ for 2 h. Then the supernatant was collected, and the melanin content was measured at 405 nm and normalized to the control.

2.7. Real-time quantitative polymerase chain reaction (RT-qPCR)

The total RNA from cultured cells was extracted with TRIzol reagent and reverse-transcribed to cDNA using the M-MLV Reverse Transcriptase (Promega, USA). The primers for genes were synthesized by Sangon (China). RT-qPCR was then performed using the GoTaq® qPCR Master Mix (Promega, USA) and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, USA). Relative mRNA levels were calculated using the $2^{-\triangle\triangle}$Ct method after normalization to the level of β-actin.

2.8. Fontana-Masson staining

The cells were fixed in 4% paraformaldehyde and stained with Fontana ammoniacal silver solution for 24 h at room temperature. Then, the cells were stained with hypo solution and neutral red each for 5 min. Images were obtained using a microscope (Mshot MDX10, China).

2.9. Study design and study population of the clinical trial

Patients with facial melasma and sensitive skin were recruited and then randomly allocated to either the placebo or experimental group using a computerized randomization protocol. Both the researchers and participants were blinded to the group assignments throughout the trial. The treatment period lasted 8 weeks. During the trial, all patients received the product or placebo, and sunscreen (SPF 50). All subjects were asked to apply the provided emulsion to the whole face twice daily. At each visit, subjects were assessed for ITA°, MI, area proportion of melasma, mean optical density of melasma, stratum corneum hydration (SCH), and TEWL by corresponding instruments. The mMASI score, skin tone, erythema, facial telangiectasia, dryness, and scales were assessed by a trained dermatologist.

2.10. Statistical analysis

The data, presented as mean values with standard deviations (mean ± SD), were analyzed statistically using IBM SPSS Statistics version 26. Both the Student's *t* test and one-way analysis of variance (ANOVA) were employed to assess differences between groups. The significance thresholds were designated as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Figures were created using GraphPad Prism version 8.0.

3. Results

3.1. Oxyresveratrol modulates key pathways of hyperpigmentation and sensitive skin

A total of 9693 overlap targets for hyperpigmentation and sensitive skin were obtained. The targets of 15 skin-whitening compounds were collected and subsequently intersected with the overlap targets. The top compound with the most intersecting targets was found to be

oxyresveratrol, suggesting its potential effects on sensitive skin and hyperpigmentation. The results showed that oxyresveratrol regulated most pathways relative to sensitive skin and hyperpigmentation, involving immune, barrier function, nervous, and melanin (Figure 1).

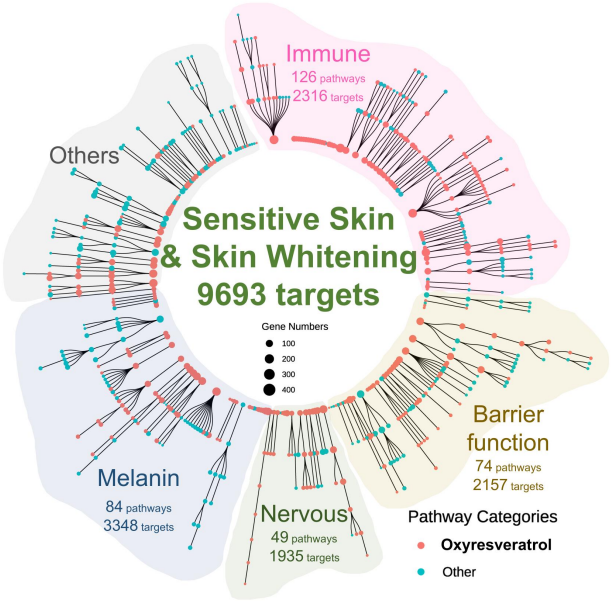

Figure 1. Oxyresveratrol modulates key pathways of sensitive skin and skin whitening.

3.2. Oxyresveratrol inhibited the activity of human tyrosinase

Oxyresveratrol, resveratrol, and phenylethyl resorcinol effectively inhibited the oxidation of *L*-DOPA by human tyrosinase *in vitro*, with IC_{50} values of 2.27 μg/mL, 13.06 μg/mL, and 18.97 μg/mL, respectively. In contrast, the inhibitory ability of glabridin was unexpectedly low, with only a 23.88% inhibition rate at the saturation concentration of 60 μg/mL (Table 1). In addition to IC_{50} values, the maximum inhibition rate of oxyresveratrol was up to 98.02%, higher than resveratrol and phenylethyl resorcinol and far more than glabridin. These results indicated that oxyresveratrol is a potent inhibitor of human tyrosinase.

Table 1. Inhibitory effects of oxyresveratrol, resveratrol, phenylethyl resorcinol, and glabridin on human tyrosinase activity.

NO.	Compounds	IC_{50} (μg/mL)	Max Inhibition Rate (%)
1	Oxyresveratrol	2.27	98.02
2	Resveratrol	13.06	93.78
3	Phenylethyl Resorcinol	18.97	94.65
4	Glabridin	> 60	≥ 23.88

3.3. Oxyresveratrol inhibited melanin production through down-regulating the MC1R/cAMP/MITF signaling pathway in B16F10 cells

The impact of oxyresveratrol on melanogenesis was further confirmed in B16F10 cells. A significant reduction in melanin content was observed, with decreases of 7.2%, 39.0%, and 28.5% at oxyresveratrol concentrations of 5, 10, and 15 μg/mL, respectively, compared with the control (Fig. 2B). RT-qPCR analysis demonstrated significant down-regulation of *MC1R*, *MITF*, and MITF-targeted genes by oxyresveratrol treatment, including *PMEL*, *TYR*, *TRP-1*, and *TRP-2* (Fig. 2C). These results suggested that oxyresveratrol inhibited melanin synthesis in B16F10 cells by down-regulating the *MC1R/cAMP/MITF* signaling pathway.

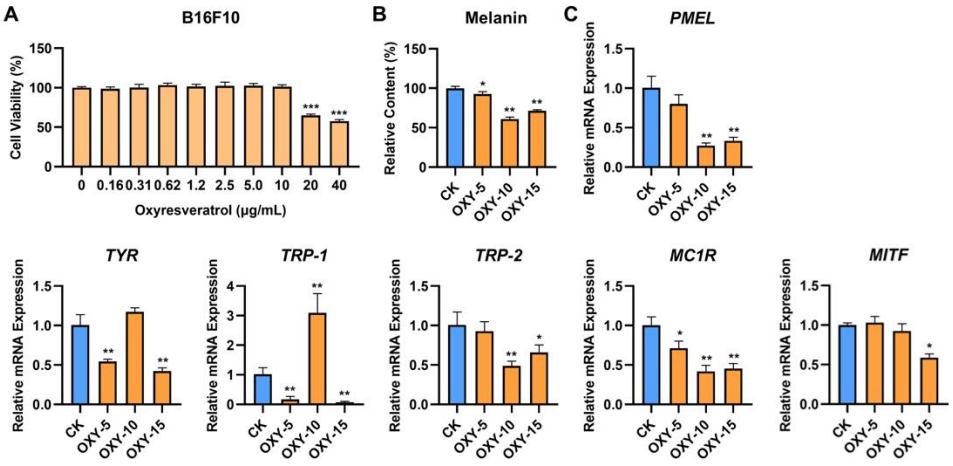

Figure 2. Oxyresveratrol inhibited melanin production through down-regulating the MC1R/cAMP/MITF signaling pathway in B16F10 cells. (A) The effect of oxyresveratrol on the viability of B16F10 cells. (B) The melanin content of B16F10 cells with oxyresveratrol treatment. CK, solvent control. OXY-5, 5 μg/mL oxyresveratrol group. OXY-10, 10 μg/mL oxyresveratrol group. OXY-15, 15 μg/mL oxyresveratrol group. (C) The effect of oxyresveratrol on the expression of seven melanogenesis-related genes in B16F10 cells.

3.4 Oxyresveratrol suppressed melanin transfer from B16F10 to HaCaT cells through inhibiting dendrite formation and melanosome transport via reducing GTPases and kinesin levels

The effect of oxyresveratrol on melanin transfer was explored using a co-culture system of B16F10 and HaCaT cells. Treatment with 10 μg/mL oxyresveratrol resulted in significant reductions in melanin content within B16F10 cells and in the transfer to HaCaT cells, with decreases of 72.0% and 85.9%, respectively (Fig. 3A–B). The descent degree of melanin content in HaCaT cells is higher than that in single-cultured B16F10 cells (Fig. 2B and Fig. 3B), suggesting that oxyresveratrol suppressed melanin transfer from B16F10 to HaCaT cells.

Microscopic analysis further revealed that oxyresveratrol significantly reduced the proportion and average length of melanocyte dendrites by 60.8% and 23.2%, respectively, compared to the control group (Fig. 3C). These findings suggested that oxyresveratrol disturbed melanocyte dendrite formation.

Further investigation using RT-PCR revealed that oxyresveratrol significantly down-regulated the expression of genes involved in dendrite development and melanosome transport, encoding GTPases CDC42, RAB17, RAB11B, and RAC1, motor protein KIF5B, and the transport-related tripartite complex RAB27A-MLPH-MYO5A (Fig. 3D). These results revealed that oxyresveratrol suppressed melanin transfer through inhibiting melanocyte dendrite

formation and melanosome transport via down-regulating the expression of *CDC42*, *RAB17*, *RAB11B*, *RAC1*, and *RAB27A-MLPH-MYO5A*.

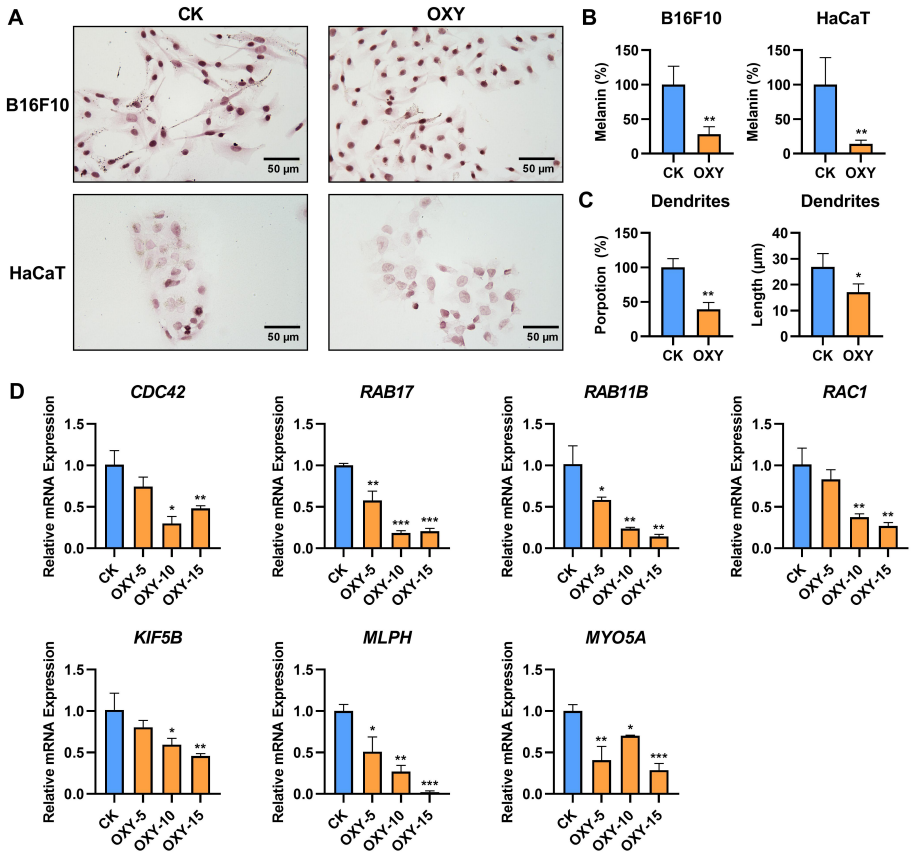

Figure 3. The effect of oxyresveratrol on melanin transfer in the co-culture of B16F10 and HaCaT cells. (A) The micrograph of B16F10 and HaCaT cells with or without oxyresveratrol treatment. CK, solvent control. OXY, treatment with 10 μg/mL oxyresveratrol. Melanin and argyrophilic cell granules were stained in black, and the nucleus was stained in red. (B) The melanin content of B16F10 and its transfer to HaCaT cells. (C) The proportion and length of melanin dendrites in B16F10 cells. (D) The effects of oxyresveratrol on the expression of eight genes associated with dendrite development and melanosome transport in B16F10 cells.

3.5. Oxyresveratrol improved facial melasma after 8-week treatment

A prospective, randomized, double-blind, and parallel-controlled trial was conducted to further explore the efficacy and safety of oxyresveratrol in melasma treatment. Between January 2024 and March 2024, a total of 70 female subjects aged 24–50 years with visible facial melasma and sensitive skin were enrolled in this study, of whom 35 were randomized to group A (placebo) and 35 to group B (1% oxyresveratrol). All the subjects completed the treatment phase. Baseline characteristics were well-balanced between the two groups.

The effectiveness of oxyresveratrol on melasma management was principally assessed by the mMASI score. Application of the 1% oxyresveratrol emulsion significantly decreased the mMASI score of patients after 2, 4, and 8 weeks, compared to placebo or baseline (Fig. 4A–B). Besides, the ITA°, MI, mean optical density of melasma, and visual assessment of skin tone in the melasma area were all significantly improved after 8 weeks of oxyresveratrol

treatment, compared to the placebo or baseline (Fig. 4C–L). These results indicated that oxyresveratrol exhibited effective clinical effects on facial melasma.

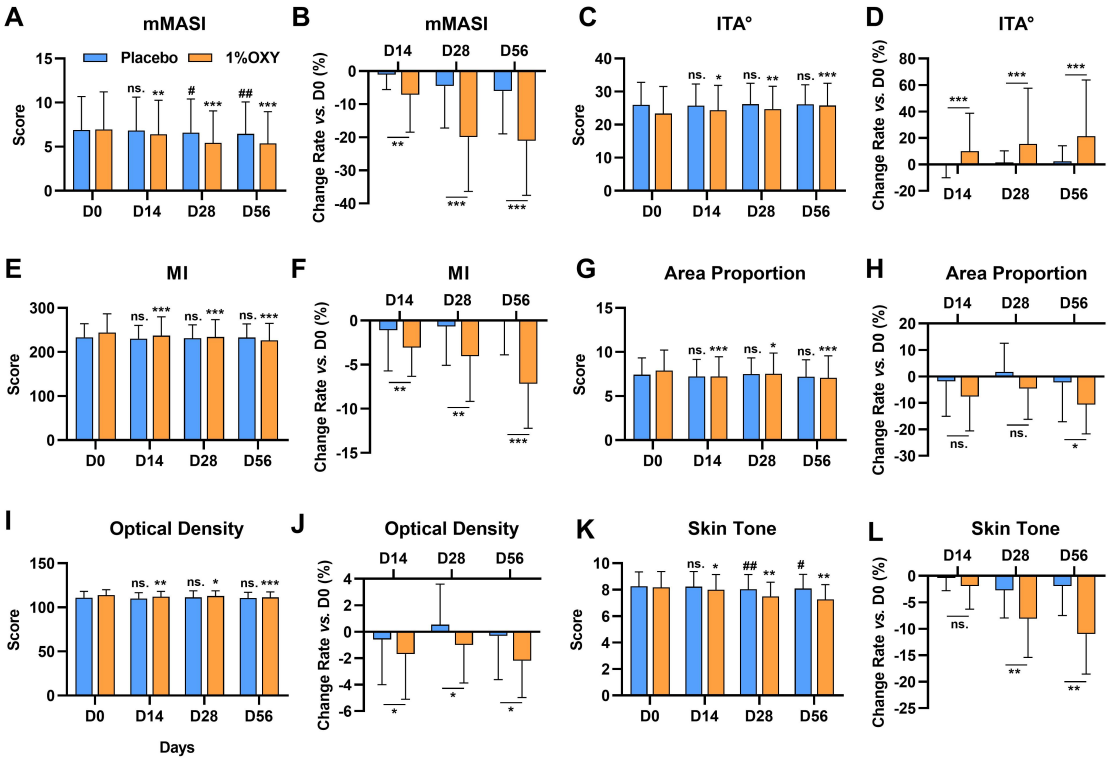

Figure 4. The objective and subjective assessments of melasma severity during the 56-days treatment period. The score of mMASI (A), ITA° (C), MI (E), area proportion (G), mean optical density (I), and skin tone (K) in the melasma area at days 0, 14, 28, and 56. The change rate of mMASI (B), ITA° (D), MI (F), area proportion (H), mean optical density (J), and skin tone (L) in the melasma area at days 14, 28, and 56.

3.6. Oxyresveratrol improved skin barrier function on sensitive skin during the 8-week trial

The effect of oxyresveratrol on sensitive skin was principally assessed by the TEWL and erythema value. Although the results of the visual assessment by investigators showed that no significant differences in TEWL and SCH were observed between the oxyresveratrol and placebo groups during the treatment period, both groups showed significant improvements in these parameters after 2, 4, and 8 weeks of application compared to baseline (Fig. 5A–D). What's more, the application of 1% oxyresveratrol emulsion significantly improved the erythema, facial telangiectasia, dryness, and scale after 8 weeks of treatment, compared to the placebo or baseline (Fig. 5E–L). These results indicated that the 1% oxyresveratrol emulsion was non-irritating to the skin and effective in improving the symptoms of sensitive skin.

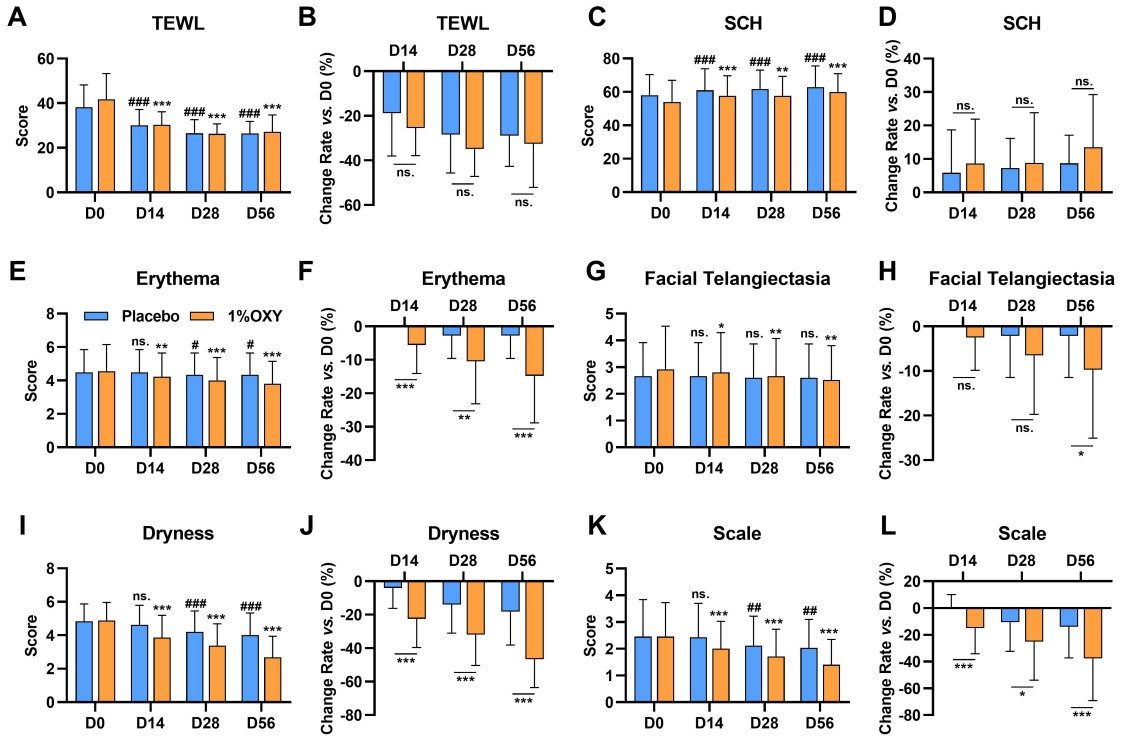

Figure 5. The objective and subjective assessments of skin barrier function during the 56-day treatment period. The score of skin conditions including TEWL (A), SCH (C), erythema (E), facial telangiectasia (G), dryness (I), and scale (K) at days 0, 14, 28, and 56. The change rate of TEWL (B), SCH (D), erythema (F), facial telangiectasia (H), dryness (J), and scale (L) at days 14, 28, and 56.

4. Discussion

Melasma is a worldwide chronic skin disorder with hyperpigmentation in light-exposed areas, especially in women, which significantly impacts facial appearance and causes psychological distress in patients [1-5]. However, current agents for melasma face limitations due to irritation risks, particularly in individuals with sensitive skin, with resulting suffering and condition aggravation for patients [3]. This study demonstrates oxyresveratrol's potential as a novel melasma treatment by targeting multiple pathogenic mechanisms. It outperformed other resorcinol-based compounds (e.g., glabridin) in inhibiting human tyrosinase and suppressed the *MC1R/cAMP/MITF* pathway, reducing melanogenic gene expression (*TYR*, *TRP-1/2*). Additionally, oxyresveratrol disrupted melanosome transport by downregulating dendrite-related proteins (CDC42, RAC1) and the RAB27A-MLPH-MYO5A complex. Clinically, 1% oxyresveratrol significantly improved melasma severity in patients with sensitive skin after 8 weeks, while alleviating erythema and barrier dysfunction without irritation. Unlike conventional depigmenting agents, oxyresveratrol uniquely combines anti-melanogenic, anti-inflammatory, and barrier-repairing effects, addressing both hyperpigmentation and skin sensitivity—a critical advantage given melasma's association with barrier impairment. These findings support its development as a safer, multitargeted alternative to melasma therapies.

5. Conclusion

In conclusion, these results provided evidence for the safety and efficacy of oxyresveratrol in treating melasma and the underlying mechanism. Specifically, 1% oxyresveratrol attenuated the facial melasma severity degree in 2 weeks and showed no skin irritation even on sensitive skin. It suppressed melanin accumulation through inhibiting melanogenesis, dendrite development, and melanosome transport via regulating the MC1R/cAMP/MITF signaling pathway. This study proposed a novel, safe, and effective treatment for melasma and revealed the inhibitory effect of oxyresveratrol on both melanin synthesis and transfer.

6. References

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