"Muscle relaxation-like" effect and skin well-being mechanism : insights from neurocosmetics

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Abstract

Objective: The study aims to evaluate the anti-aging effect and mechanisms of action of Inula japonica Thunb extract（IJT）rich in inulinlactone and caffeoylquinic acid from a neurocosmetic perspective.

Methods: First, a human skeletal muscle collagen gel model was established to evaluate the relaxant effect of Inula britannica flower extract (ITJ) on skeletal muscles. Second, SH-SY5Y neuroblastoma cells were used to assess the effects of ITJ on the expression of glutamate decarboxylase 67 (GAD67), GABAA receptor (GABAAR), and GABAB receptor (GABABR) through real-time PCR. Subsequently, the impacts of ITJ on protein expression of GABA, GABAAR, and GABABR were evaluated via immunohistochemical staining and quantitative analysis. Finally, clinical assessments were conducted to examine the improvement effects of ITJ on dynamic eye wrinkles, skin elasticity, and firmness.

Results: At a concentration of 3.13×10^{-3}mL/mL, ITJ significantly promoted skeletal muscle cell relaxation compared to the control group ($P < 0.05$). Additionally, mRNA expression levels of GAD67 and GABAAR were markedly increased in the ITJ-treated group ($P < 0.05$). Quantitative immunohistochemical staining results demonstrated significantly elevated expression of GABA, GABAAR and GABABR ($P < 0.01$). Furthermore, clinical evaluations revealed that ITJ rapidly improved the appearance of crow's feet and enhanced periocular skin elasticity and firmness.

Conclusion: This study integrates sustainable plant ingredients with neurocosmetics, drawing inspiration from traditional chinese medicine. It elucidates the mechanism by which plants promote muscle relaxation through the regulation of the neurotransmitter γ-aminobutyric acid and its associated biomarkers, validating its beneficial effects on skin health and offering a novel perspective on the application of plant-based ingredients in neurocosmetics.

Key words: Neurocosmetics; GABA; Muscle Relaxation; Anti-aging; Traaditional Chinese Medecine

1. Introduction

The skin, as a remarkable organ, manages the relationships between us and the outside world. With advancements in understanding its physiological functions, the scientific community has increasingly recognized the skin as a sophisticated intelligent communication system, maintaining close interconnections with the nervous and immune systems [1, 2]. The skin is no longer viewed as an isolated organ but rather as a critical interface integrating neural,

immune, and endocrine pathways to sustain cutaneous physiological homeostasis [3]. This paradigm shift, which positions the skin within neuro-immuno-cutaneous system for functional investigation, has emerged as one of the most pivotal domains in skin biology research [4]. In this context, neurocosmetics has gradually become a prominent interdisciplinary focus.

The concept of neurocosmetics was first proposed in 2000 by Professor Misery from the University of Brest Medical School in France, defined as "referring to them as ” not absorbed products applied on the skin, exhibiting activity on the cutaneous nervous system or in general effects on the skin mediators" [5]. It regulates skin functions through topical modulation of the cutaneous nervous system. Among the over 200 known neurotransmitters, approximately 25 have been identified in the skin [4]. The binding of neurotransmitters to their receptors induces modifications in cellular characteristics and skin function regulation. In particular, the regulation of neurotransmitters such as γ-aminobutyric acid (GABA) and acetylcholine to maintain skin homeostasis and delay aging has become a significant branch in cosmetic anti-aging research, owing to its rapid onset of effects.

Acetylcholine is the primary neurotransmitter released by motor nerve endings, acting as an excitatory neurotransmitter that mediates skeletal muscle contraction [6]. GABA, a typical inhibitory neurotransmitter, collaborates with acetylcholine to influence muscle relaxation and contraction by regulating intracellular metabolic processes and calcium ion levels [7]. The synergistic action of these two neurotransmitters mediates skeletal muscle contraction, thereby contributing to the smoothing of wrinkles. Neurocosmetic ingredients functioning as neurotransmitter modulators-such as Syn-Ake[8] and Acetyl Hexapeptide-8 [9]-are increasingly being discovered to achieve anti-wrinkle. Botanical ingredients have emerged as a research focus in this field due to their natural origin, ecologically sustainable properties, and multi-target/multi-pathway mechanisms of action.

Inula japonica Thunb, the dried capitulum of *Inula japonica Thunb*. or *I. britannica L.*, serves as the sovereign herb in the classical Chinese medicinal formula Xuanfu Daizhe Decoction（XDD） [10]. With 1,800 years of clinical application history, XDD has been widely recognized for its therapeutic effects in relaxing smooth muscles and treating gastric spasms [11]. Notably, XDD was listed in the Directory of Ancient Classic Prescriptions (First Batch) by China's National Medical Products Administration [12], owing to its exceptional efficacy in managing gastric spasms. Inspired by traditional medicine, this study explores the potential of Inula japonica Thunb as a neurocosmetic ingredient and investigates its underlying mechanisms of action.

2. Materials and Methods

2.1 Study on the effect of promoting relaxation of HSkMC Cells

2.1.1 Experimental materials

The cell experiments utilized Inula japonica Thunb extract (IJT), characterized as a brownish-yellow clarified liquid. HPLC analysis revealed the following phytochemical composition: 0.11 mg/mL 1,5-Di-O-caffeoylquinic acid, 96.7 μg/mL1-O-Acetylbritannilactone.

Human skeletal muscle cells (HSkMC) were employed in this study, subcultured in our laboratory prior to experimentation.

2.1.2 Experimental methods

2.1.2.1 Cytotoxicity Assessment of IJT on HSkMC Cells

（1）Test Substance Preparation

The stock solution of IJT was diluted 10-fold with culture medium to prepare the first test concentration (0.1mL/mL). Subsequent 2-fold serial dilutions were performed using culture medium to achieve final concentrations.

（2）Cell Culture and Treatment

① Cryopreserved HSkMC cells were thawed, subcultured, and adjusted to a density of 5×10^4 cells/mL. The cell suspension was seeded into 96-well plates (90 μL/well) and cultured for 24h at 37°C under 5% CO_2.

② 10 μL of test samples or culture medium (control) was added to each well, followed by 48h incubation prior to CCK-8 assay.10 μL of CCK-8 reagent was added to each well, and the plates were incubated in the dark for 1h at 37°C with 5% CO_2.

③ Optical density (OD) was measured at 450 nm using a microplate reader. Cell viability was calculated based on OD values.

（3）Analytical Method

Cell viability (%) = [(OD of tset well)-(OD of blank well)/(OD of control well)-(OD of blank well)]×100%

2.1.2.2 Relaxation Assessment of IJT on HSkMC Cells

（1）Cell Culture and Treatment

HSkMC cells were resuspended in a cell culture medium-collagen mixture, with approximately 50,000 cells seeded per well of a 24-well plate, followed by incubation at 37° C for 1h.

Each well was supplemented with 1 mL of complete medium and cultured for 48h.

After adding the corresponding test samples and incubating for 2 h, collagen matrices were mechanically released using pipette tips, and contraction was monitored over 48h.

（2）Analytical Methods

Gel matrix diameter was measured to calculate contraction length and percentage. Results are expressed as mean±standard deviation (X±SD), with statistical analysis performed using Student's two-tailed t-test.

Contraction Percentage (%) = [(15 mm-Final gel diameter) / 15 mm] × 100

2.2 Study on the mechanism of GABA signal regulation of SH-SY5Y cells

2.2.1 Experimental materials

The nucleotide sequences of *GABARA mRNA, GABARB mRNA, GAD67 mRNA*, and *β-ACTIN mRNA* were retrieved from the NCBI Nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/). Forward and reverse primers were designed using the Primer-BLAST tool. (Table 1)

The human neuroblastoma cell line SH-SY5Y was utilized in this study, subcultured in our laboratory prior to experimentation.

Table 1. RT-qPCR primer sequence table

Gene	primer	Sequence (5'-3')	product(bp)	GenBank
GABARA	Forward primer	GGGCGAGAAGATCCGAAAGA	180	NM_007278.2
	Reverse primer	AACAAGGCATCCTCAGCTCG		
GABARB	Forward primer	GCAGATCCGCAACGAGTCA	143	NM_005458.8
	Reverse primer	CGCCTCCAAACACCATCAAG		
GAD67	Forward primer	GCGTGTTGAGTACGTTCTGG	108	M81883.1

	Reverse primer	TGACCGCCTCTGGAGCTTTA		
β-ACTIN	Forward primer	CTCACCATGGATGATGATATCGC	168	NM_001101.5
	Reverse primer	CACATAGGAATCCTTCTGACCCA		

2.2.2 Experimental methods

2.2.2.1 Real-time RT PCR

（1）Test Substance Preparation

Experiments were conducted with blank control and test groups, each containing six replicates. The test group received a concentration of 3.13×10^{-3} mL/mL, selected based on prior skeletal muscle cell relaxation efficacy studies.

All procedures utilized 24-well plates, with cells seeded at a density of 6×10^{5} cells/mL. After 48 h of culture, test samples were added and incubated for 4 h.

（2）Total RNA Extraction

① 0.5 mL TRIzol was added to each well of the 24-well plate. Lysates were gently scraped, transferred to 1.5 mL microcentrifuge tubes, and incubated at room temperature for 20 min, followed by centrifugation (4°C, 12,000rpm, 10min).

② 0.2 mL chloroform was added, tubes were vigorously shaken for 15 s, incubated for 3 min at room temperature, and centrifuged (4°C, 12,000rpm, 15min).

③ The aqueous phase was transferred, mixed with 0.5 mL isopropanol, incubated at room temperature for 30 min, and centrifuged (4°C, 12,000rpm, 10min).

④ Supernatant was discarded. RNA pellets were washed with 1 mL 75% ethanol, resuspended by vortexing, and centrifuged (4°C, 7,500rpm, 5min).

⑤ Pellets were air-dried briefly, dissolved in 15 μL DEPC-treated water, and stored at -80°C.

（3）Real-time RT PCR

① Reverse transcription and amplification were performed using the One Step TB Green™ PrimeScript™ RT-PCR Kit, with fluorescence monitored in real-time.

② Reaction mixture (20 μL): 2 μL RNA, 10 μL 2× One Step TB Green RT-PCR Buffer, 0.8 μL each of 10 pmol forward/reverse primers.

③ Thermal profile: Reverse transcription at 42°C for 5 min; initial denaturation at 95°C for 10 s; 40 cycles of 95°C for 5 s and 60°C for 34 s.

（4）Analytical Methods

Data were analyzed using SDS v1.4 software. Relative quantification was calculated via the $2^{-\Delta\Delta CT}$ method, with β-ACTIN as the endogenous control. Results are expressed as mean ± SD. Statistical significance was determined by one-way ANOVA using GraphPad Prism 7.0, with $P < 0.05$ (*) and $P < 0.01$ (**) indicating significance.

2.2.2.2 Immunohistochemical (IHC) Staining

（1）IHC Staining

① Multiplex IHC staining of SH-SY5Y neuroblastoma cells was performed using the Opal7-Color Automation IHC Kit. After heat-induced epitope retrieval (HIER), slides were blocked with PerkinElmer Antibody Diluent/Block Buffer, followed by 1 h incubation at room temperature with primary antibodies: anti-GABBR2, anti-GABA, and anti-GABRA1 antibodies.

② Slides were washed in TBST and incubated with HRP-conjugated Ms+Rb secondary antibodies for 10 min at room temperature. Opal fluorophores (1:100 dilution) were applied to slides for 10 min, followed by microwave-mediated antibody stripping to remove primary/secondary antibody complexes.

③ Nuclei were counterstained with DAPI (0.5 μg/mL) for 10 min at room temperature. Coverslips were mounted using BDHM-BD-FM-1 mounting medium. Images were acquired on a Vectra Polaris™ Automated Quantitative Pathology System (PerkinElmer) and analyzed with inForm® 2.8.0 software to quantify the intercellular positive expression area ratio.

（2）Analytical Methods

Data are presented as mean ± SD. Statistical significance was determined by unpaired two-tailed Student's t-test using GraphPad Prism 9.0, with $P < 0.05$ (*) and $P < 0.01$ (**) indicating significance.

2.3 Clinical Efficacy Evaluation

（1）Experimental methods

Test products: IJT Anti-Wrinkle Mask (test group); Vehicle Mask (control group)

This study enrolled 20 healthy female Chinese volunteers aged 30-55 years with crow's feet wrinkles (Grade 2-4) and bilateral cheek elasticity F4 index >6. After obtaining informed consent, all 20 participants completed the clinical trial.

The experiment was conducted in accordance with relevant anti-wrinkle efficacy testing standards [13,14]. Volunteers applied half-sheet masks to each side of the face for 20 minutes:, Test group: IJT Anti-Wrinkle Mask (left/right face, randomized). Control group: Vehicle Mask (contralateral side).

Anti-wrinkle efficacy parameters:

① Crow's feet wrinkle area & average depth: Evaluated using the EvaFACE® Rapid Optical Imaging System (Courage+Khazaka) before and after application.

② Periorbital elasticity & firmness: Measured with the Cutometer® dual MPA580 (Courage+Khazaka) via suction elongation methodology.

（2）Analytical methods

Post-treatment differences (Δ = post-treatment value-baseline value) were calculated for both groups. Statistical analysis compared Δ values between groups using SPSS Statistics 25. Normally distributed data: Unpaired two-tailed Student's t-test (α =0.05), Non-normal distributions: Mann-Whitney U test.

Results are expressed as mean ± standard deviation (SD), compared with the initial value, *$p < 0.05$, **$P<0.01$, compared with the control group, $^{\#}p < 0.05$, $^{\#\#}P<0.01$.

3. Results

3.1 Study on the effect of promoting relaxation of HSkMC Cells

3.1.1 Cytotoxicity Assessment of IJT on HSkMC Cells

The effects of IJT on HSkMC cell viability are presented in Table 2. Results demonstrated that the maximum safe concentration determined to be 6.25×10^{-3} mL/mL.

Table 2. Results of the Cytotoxicity Effects of IJT on HSkMC Cells

Gurop	Concentration （mL/mL）	Cell viability （%）
IJT	1.00×10^{-1}	$2.55\pm0.09^{**}$
	5.00×10^{-2}	$9.73\pm1.28^{**}$

	2.50×10^{-2}	38.78±4.12**
	1.25×10^{-2}	70.07±3.07**
	6.25×10^{-3}	94.84±4.9
	3.13×10^{-3}	106.74±2.54
Blank control	/	100.00±10.66
Solvent control (butanediol)	/	101.98±0.42

3.1.2 Relaxation Assessment of IJT on HSkMC Cells

The effects of IJT on HSkMC cell contractility are summarized in Table 3. Results indicate that all tested concentrations of IJT reduced the cell contraction rate, demonstrating a dose-dependent muscle relaxation effect. Notably, the concentration of 3.13×10^{-3} mL/mL showed a significant difference compared to the blank control group ($P < 0.05$), with a contraction rate of 12.89%. Therefore, 3.13×10^{-3} mL/mL IJT was selected for subsequent mechanistic studies on skeletal muscle cell relaxation.

Table 3. Results of the Contractility Effects of IJT on HSkMC Cells

Gurop	Concentration （mL/mL）	Terminal diameter （mm）	Diameter contraction length （mm）	Contractility （%）
IJT	6.25×10^{-3}	12.47±0.84	2.53±0.84	16.89±5.59
	3.13×10^{-3}	13.07±0.4*	1.93±0.40*	12.89±2.69
	1.56×10^{-3}	12.6±0.56	2.40±0.56	16.00±3.71
Blank Group	/	11.97±0.55	3.03±0.55	20.22±3.67
Positive control	/	14.63±0.12**	0.37±0.12**	2.44±0.77

3.2 Study on the mechanism of GABA signal regulation of SH-SY5Y cells

3.2.1 IJT activates the mRNA expression of GABARA， GABARB and GAD67 on SH-SY5Y cells

Experimental results demonstrated that following 2-hour incubation of SH-SY5Y cells with IJT, GABARA mRNA expression was significantly increased compared to the blank control group ($P < 0.01$). GAD67 mRNA expression showed a significant elevation relative to the blank control group ($P < 0.01$). GABARB mRNA expression exhibited an upward trend but did not reach statistical significance compared to the blank control group ($P > 0.05$). (Table 4)

Table 4. IJT activates the mRNA expression on SH-SY5Y cells

Gurop	Concentration （mL/mL）	GABARA mRNA	GABARB mRNA	GAD67 mRNA
IJT	3.13×10^{-3}	50.85±7.15**	1.52±0.31	4.40±0.92**
Blank control	/	3.78±1.78	1.28±0.25	1.57±0.56

Note: Compared with the blank control group, *P<0.05, **P<0.01, n=6

3.2.2 IJT activates the expression of GABA, GABARA and GABARB on SH-SY5Y cells

Experimental results demonstrated that after a 2-hour incubation of SH-SY5Y cells with IJT, GABA expression was significantly upregulated compared to the blank control group ($P < 0.01$). GABARA expression exhibited a significant increase relative to the blank control group ($P < 0.01$). GABARB expression showed marked elevation with statistically significant differences versus the blank control group ($P < 0.01$). (Table 5)

Table 5. IJT activates the expression on SH-SY5Y cells

Gurop	Concentration （mL/mL	GABA	GABARA	GABARB
IJT	3.13×10^{-3}	$21.65\pm8.63^{**}$	$67.55\pm8.70^{**}$	$51.73\pm10.81^{**}$
Blank control	/	9.09±1.21	22.87±7.98	23.23±3.63

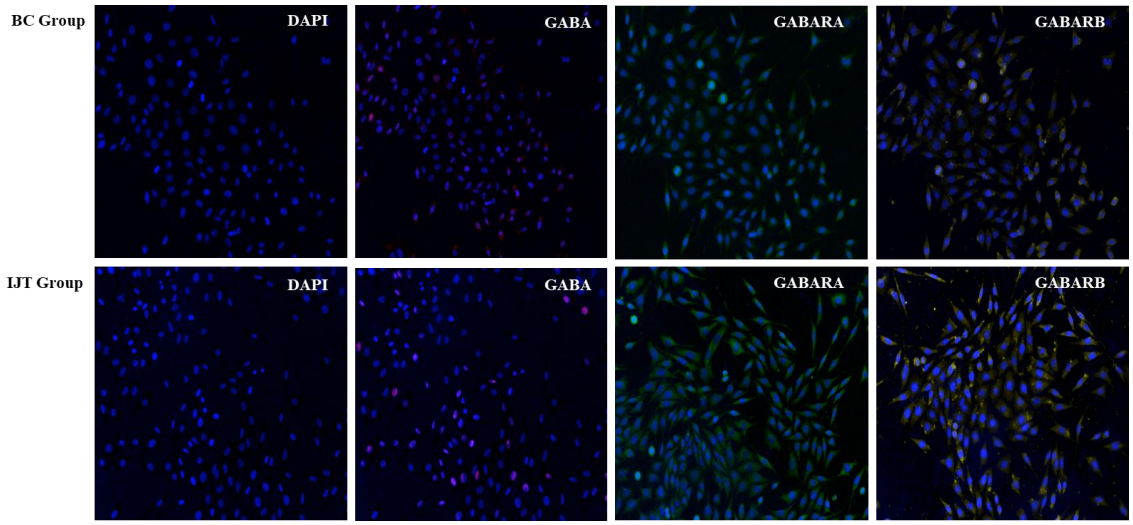

Figure 1. Experimental results of IJT activating GABA and target protein IHC staining on SH-SY5Y cells

3.3 Clinical Efficacy Evaluation

3.3.1 Experiment on improving canthus wrinkles with IJT

The canthus wrinkles area results demonstrated that compared with baseline, the test group showed a significant 9.94% reduction in wrinkle area 20 minutes after application ($P < 0.01$). When compared with the control group, the test group exhibited a significantly superior change trend in 20 minutes after product application ($P < 0.05$).

Table 6. Experiment on improving canthus wrinkles area with IJT

Group	canthus wrinkles area		Improvement rate of canthus wrinkle area relative to the initial value (%)
	Before use	After 20min	
Test	24.290	21.876	$-9.94^{**\#}$
Control	23.334	22.782	-2.37

The canthus wrinkles depth results demonstrated that compared with baseline, the test group showed a significant 4.72% reduction in wrinkle depth 20 minutes after application ($P < 0.01$). When compared with the control group, the test group exhibited a significantly superior change trend in 20 minutes after product application ($P < 0.01$).

Table 7. Experiment on improving canthus wrinkles depth with IJT

Group	canthus wrinkles depth		Improvement rate of canthus wrinkle depth relative to the initial value (%)
	Before use	After 20min	
Test	-0.036	-0.034	-4.72**##
Control	-0.032	-0.033	2.18

3.3.2 Experiment on improving eye elasticity with IJT

The eye elasticity results demonstrated that compared with baseline, the test group showed a significant 4.65% improvement in elasticity 20 minutes after application ($P<0.01$). When compared with the control group, the test group exhibited a significantly superior change trend in 20 minutes after product application ($P<0.01$).

Table 8. Experiment on improving eye elasticity with IJT

Group	eye elasticity		Improvement rate of eye elasticity relative to the initial value (%)
	Before use	After 20min	
Test	0.561	0.587	4.65**#
Control	0.563	0.567	0.85

The eye tightness results demonstrated that compared with baseline, the test group showed a significant 3.61% improvement in tightness 20 minutes after application ($P<0.01$). When compared with the control group, the test group exhibited a significantly superior change trend in 20 minutes after product application ($P<0.01$).

Table 9. Experiment on improving eye tightness with IJT

Group	eye tightness		Improvement rate of eye tightness relative to the initial value (%)
	Before use	After 20min	
Test	11.84	11.42	-3.61**#
Control	11.85	11.83	-0.14

4. Discussion

This study explores the potential application and mechanism of action of IJT as a neurocosmetic ingredient. The results show that IJT, rich in 1-O-acetyl inulinolide and 1,5-dicaffeoylquinic acid, significantly promotes skeletal muscle relaxation at a concentration of 3.13×10^{-3} mL/mL compared to the control group ($P<0.05$). On one hand, IJT acts as a regulator of neurotransmitter release and significantly promotes the expression of GABA ($P<0.01$). On the other hand, it acts as an agonist of GABA receptors, significantly promoting the expression of GABAAR and GABABR proteins ($P<0.01$). In addition, IJT also significantly promotes the expression of GAD67 mRNA ($P<0.05$).

GABA is a typical inhibitory neurotransmitter, and its increased release can activate GABAAR and GABABR [15]. GABAAR is a ligand-gated chloride ion channel. Upon activation, chloride ion influx leads to hyperpolarization of the presynaptic membrane, thereby inhibiting the opening of voltage-gated calcium channels (VGCC), reducing calcium ion influx, and further decreasing the excitability of nerve terminals [16]. GABABR is a G protein-coupled receptor. Upon activation, it directly inhibits the opening of VGCC in the presynaptic membrane,

reducing calcium ion influx [17]. Baclofen, a GABABR agonist, is widely used to treat muscle spasms, and its mechanism of action is mainly through the inhibition of the release of excitatory neurotransmitters in the spinal cord via GABABR [18]. Although its primary site of action is the central nervous system, clinical evidence shows that the activation of GABABR can reduce excessive muscle contraction. Previous studies have already demonstrated the role of acetylcholine in regulating skeletal muscle contraction [19], but there have been few studies on the role of GABA in regulating skeletal muscle contraction. This study suggests the potential application of GABA in promoting skeletal muscle relaxation.

Inula japonica Thunb. mainly contains sesquiterpene lactones, phenolic acids, and terpenoids, among which sesquiterpene lactones are characteristic components of Inula japonica Thunb. [20]. 1-O-Acetyl inulinolide is a typical sesquiterpene lactone, and studies have shown that sesquiterpene lactones can reduce intracellular calcium ion concentration by inhibiting L-type calcium channels, thereby affecting smooth muscle contraction [21]. 1,5-Dicaffeoylquinic acid is a derivative of caffeic acid, and studies have shown that derivatives of caffeic acid can reduce calcium ion influx, affecting neuronal excitability [22], and can also protect neurons by reducing calcium overload caused by oxidative stress through antioxidant mechanisms [23]. This study suggests that sesquiterpene lactones and caffeic acid derivatives should be highlighted as neurocosmetic ingredients.

In this study, a clinical evaluation was conducted using facial masks. The results showed that after applying a mask containing IJT for 20 minutes, the canthus wrinkles area was significantly reduced by 9.94% ($P < 0.01$), the canthus wrinkles depth was significantly reduced by 4.72% ($P < 0.01$), eye elasticity was significantly enhanced by 4.65% ($P < 0.01$), and eye tightness was significantly improved by 3.61% ($P < 0.01$). These findings further validate the efficacy of IJT, suggesting that dosage forms promoting penetration are conducive to the action of neurocosmetic ingredients. The synergistic effect between penetration behavior and neurocosmetic ingredients warrants further investigation.

5. Conclusion

In summary, this study has revealed that IJT exerts its skeletal muscle relaxation effect by modulating multiple targets in the GABAergic pathway, and has further confirmed its beneficial effects on the skin through clinical evaluation. Meanwhile, the sesquiterpenes and caffeic acid derivatives in IJT, such as 1-O-acetyl inulinolide and 1,5-dicaffeoylquinic acid, have demonstrated great potential as neurocosmetic ingredients. Drawing inspiration from traditional medicine, this study integrates traditional medical knowledge with neurocosmetics, expands our understanding of neurocosmetic ingredients, and highlights the unique mechanisms of action of plant-based neurocosmetic components. It provides a new perspective for the application of plant ingredients in neurocosmetics.

6. References

[1] Ramot Y, Böhm M, Paus R. Translational Neuroendocrinology of Human Skin: Concepts and Perspectives. Trends Mol Med. 2021 Jan;27(1):60-74.

[2] Jin R, Luo L, Zheng J. The Trinity of Skin: Skin Homeostasis as a Neuro-Endocrine-Immune Organ. Life (Basel). 2022 May 12;12(5):725.

[3] Shastri M, Sharma M, Sharma K, Sharma A, Minz RW, Dogra S, Chhabra S. Cutaneous-immuno-neuro-endocrine (CINE) system: A complex enterprise transforming skin into a super organ. Exp Dermatol. 2024 Mar;33(3):e15029.

[4] Vito Rizzi, Jennifer Gubitosa, Paola Fini, Pinalysa Cosma. Neurocosmetics in Skincare-

The Fascinating World of Skin-Brain Connection: A Review to Explore Ingredients, Commercial Products for Skin Aging, and Cosmetic Regulation. Cosmetics. 2021,8,66.
[5] Misery, L. Les nerfs à fleur de peau. Int. J. Cosmet. Sci. 2002, 24, 111-116.
[6] Li H, Teng J, Hibbs RE. Structural switch in acetylcholine receptors in developing muscle. Nature. 2024 Aug;632(8027):1174-1180.
[7] Sibgatullina GV, Malomouzh AI. GABA in developing rat skeletal muscle and motor neurons. Protoplasma. 2020 May;257(3):1009-1015.
[8] Almeida JR, Resende LM, et al. Snake Venom Peptides and Low Mass Proteins: Molecular Tools and Therapeutic Agents. Curr Med Chem. 2017;24(30):3254-3282.
[9] Olsson SE, Sreepad B, Lee T, Fasih M, Fijany A. Public Interest in Acetyl Hexapeptide-8: Longitudinal Analysis. JMIR Dermatol. 2024 Feb 20;7:e54217.
[10] Mengru C, Kaixin W, Dongge Y,et al. Textual research on the materia medica of Inula in the prescriptions. Central South Pharmacy Journal. 2022,20(5):1142-1147.
[11] WY, XQF, LJL,et al. Quantity transfer law and influencing factors of phenolic acids in classical prescription Xuanfu Daizhe Decoction based on QAMS and standard relation. Chinese Traditional and Herbal Drugs, 2025,56(5):1596-1606.
[12] Notice of the National Administration of Traditional Chinese Medicine on the Release of the "Directory of Ancient Classic Prescriptions (First Batch). (2018, April 13). Central People's Government of the People's Republic of China. https://www.gov.cn/zhengce/zhengceku/2018-12/31/content_5429153.htm.
[13] T/GDCDC 019-2021. Test method for anti-wrinkle efficacy of cosmetics.
[14] T/ZHCA 005-2019. Test method of cosmetics affecting skin elasticity.
[15] Cuypers K, Maes C, Swinnen SP. Aging and GABA. Aging (Albany NY). 2018 Jun 13;10(6):1186-1187.
[16] Oyelese AA, Kocsis JD. GABAA-receptor-mediated conductance and action potential waveform in cutaneous and muscle afferent neurons of the adult rat: differential expression and response to nerve injury. J Neurophysiol. 1996 Oct;76(4):2383-92.
[17] Tsentsevitsky AN, Sibgatullina GV, Petrov AM, Malomouzh AI, Kovyazina IV. GABA Receptors and Kv7 Channels as Targets for GABAergic Regulation of Acetylcholine Release in Frog Neuromuscular Junction. Neurochem Res. 2024 Nov 20;50(1):25.
[18] Kent CN, Park C, Lindsley CW. Classics in Chemical Neuroscience: Baclofen. ACS Chem Neurosci. 2020 Jun 17;11(12):1740-1755.
[19] Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012 Oct 4;76(1):116-29.
[20] li L, Huanhuan L, Chunqin M, et al. Quality study of Inulae Flos based on HPLC and chemometrics. Chinese Traditional and Herbal Drugs,2021,52(6):1751-1758.
[21] Qu C, Guan X, Li C, et al. Sesquiterpene lactones improve secretory diarrhea symptoms by inhibiting intestinal Ca2+-activated Cl- channel activities directly and indirectly. European Journal of Pharmacology. 2023 Sep;955:175917.
[22] Fernandes, et al. Neuromodulation and neuroprotective effects of chlorogenic acids in excitatory synapses of mouse hippocampal slices. Sci Rep 11, 10488 (2021).
[23] Fu W, Wang H, Ren X. Neuroprotective effect of three caffeic acid derivatives via ameliorate oxidative stress and enhance PKA/CREB signaling pathway. BEHAVIORAL SCIENCES, 2017, 328: 81-86.