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"Preparation of a Saffron Oil Composition with the Ability to Regulate the Expression of Skin Cortisol"

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1. Introduction

The generation of emotions involves the coordinated action of multiple brain regions and represents the nervous system's response to external stimuli. The nervous system consists of two main parts: the central nervous system (CNS) and the peripheral nervous system (PNS). The PNS transmits neural signals from peripheral receptors to the CNS, where information from various parts of the body is integrated, processed, or stored. The amygdala serves as the core region for the generation and processing of emotions. Sensory signals can be rapidly transmitted to the amygdala via the thalamus, although this pathway may lack some details. Alternatively, sensory signals can reach the amygdala through the cerebral cortex, with this route conveying signals containing richer details. The prefrontal cortex is capable of recognizing and regulating emotions, aiding individuals in controlling their emotions and making decisions [1-3]. After an emotion arises, it triggers a series of neural responses that modulate the hormonal and immune systems, primarily involving the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system. The paraventricular nucleus of the hypothalamus synthesizes and secretes antidiuretic hormone (ADH) and corticotropin-releasing hormone (CRH). ADH and CRH act on the anterior pituitary gland, promoting the release of adrenocorticotropic hormone (ACTH). ACTH travels through the bloodstream to the adrenal cortex, where it stimulates the synthesis of glucocorticoids (such as cortisol). Excessive cortisol levels exert negative feedback regulation on the hypothalamus and pituitary gland by directly inhibiting the cleavage of POMC, thereby suppressing the production of ACTH and β-endorphin.

Stress-related emotional states can elevate serum cortisol levels through the activation of the HPA axis. Elevated cortisol concentrations are implicated in skin impairment, manifesting as increased transepidermal water loss, suppression of cholesterol, free fatty acid, and ceramide synthesis by keratinocytes, and a reduction in corneodesmosome density within the stratum corneum. These combined effects compromise the integrity and adhesive properties of the stratum corneum, thereby heightening skin sensitivity. Additionally, high cortisol levels attenuate the inflammatory response by inhibiting the production of pro-inflammatory cytokines, including interleukin-1α (IL-1α), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), which in turn impedes the early phases of cutaneous wound healing [4-6].Catecholamines, which encompass dopamine, norepinephrine, and epinephrine, serve

as crucial neurotransmitters or neurohormones in the human body. The central nervous system activates the sympathetic nervous system, prompting the release of catecholamines. These neuroactive substances then act on the innervated skin via sympathetic nerve endings. Norepinephrine and epinephrine exert their effects through adrenergic receptors, inhibiting angiogenesis within the wound bed and contributing to delayed wound repair.Additionally, catecholamines can augment cutaneous sweating [7, 8].

The skin and the nervous system share numerous hormones and neurotransmitters. The skin not only functions as a target organ for various hormones but is also capable of secreting multiple hormones itself. The skin possesses a peripheral HPA axis. Following emotional stress, keratinocytes, mast cells, sebaceous gland cells, and others secrete CRH. CRH signaling can trigger the cAMP pathway in melanocytes, leading to the production of ACTH and corticosterone, which activate various melanocyte functions such as proliferation, differentiation, cytokine production, and melanogenesis [9]. CRH has been shown to inhibit keratinocyte proliferation while stimulating their differentiation [10].The peripheral HPA axis in the skin generates POMC, which is cleaved after ultraviolet (UV) radiation exposure to produce β-endorphin. β-endorphin promotes wound healing by inducing the expression of K16 and modulating transforming growth factor-β (TGF-β) [11]. Keratinocytes are capable of synthesizing epinephrine, which acts through adrenergic receptors (ARs) present on epidermal keratinocytes and melanocytes. Epinephrine induces Ca^{2+} influx in keratinocytes via Orai1 channels, promoting keratinocyte proliferation and migration, and via TRP channels, facilitating keratinocyte differentiation [12-14]. However, overexpression of epinephrine can enhance mast cell degranulation, increase the production of anti-inflammatory cytokines, and consequently delay cutaneous wound healing [15].

Conventional approaches to mood-enhancing skincare primarily focus on alleviating consumer stress and subsequently improving skin condition through sensory experiences. These include the use of pleasant fragrances that activate olfactory receptors in olfactory epithelial cells, transmitting signals to the brain to induce positive emotions; the application of desirable textures that generate electrical currents via PIEZO2 mechanoreceptors, transmitting signals to the brain for mood modulation; and the strategic use of colors that exert psychological hint effects to regulate consumer emotions. In contrast, research investigating the modulation of the peripheral HPA axis by topical active ingredients remains limited. This study serendipitously discovered that saffron extract can mitigate stress-induced skin issues by acting on the peripheral HPA axis.

Saffron (*Crocus sativus* L.), a perennial herbaceous plant belonging to the genus Crocus within the Iridaceae family, is predominantly known for its stigmas (often referred to simply as "saffron") and is hailed as the "red gold." Valued for its coloring properties and distinct aroma, saffron is widely used as a spice. Additionally, it has a long history of use in traditional medicine for treating various ailments. The primary active constituents of saffron include crocin, crocetin, picrocrocin, and safranal. Crocin and crocetin are responsible for the characteristic yellow hue of saffron, while picrocrocin contributes to its flavor, and safranal imparts its unique aroma. Research has indicated that saffron extracts exhibit antidepressant activity, potentially mediated by the activation of serotonergic, noradrenergic, and dopaminergic systems [16].

Currently, most studies on the antidepressant effects of saffron have utilized methods such as intraperitoneal injection, oral administration, inhalation, and experiments conducted on neural cells [17, 18]. Research on the external application of saffron to the skin has predominantly centered on its anti-inflammatory and anti-photoaging properties [19, 20]. Saffron is capable of modulating emotions by influencing the HPA axis. It is noteworthy that a

cutaneous HPA axis also exists within the skin, and its impact on skin conditions has been previously summarized. The present study aims to investigate the effects of saffron on the cutaneous HPA axis and the pathways through which it acts, thereby providing a basis for the application of saffron in skincare cosmetics that aim to enhance mood.

2. Materials and Methods

2.1 Cell culture

Human immortalized keratinocytes(from DSMZ, Catalog Number: CBP60331). Fetal bovine serum (FBS) and penicillin-streptomycin mixture（from WISENT）, and DMEM medium was sourced from Gibco. The complete culture medium was formulated by combining 89% DMEM medium, 10% FBS, and 1% penicillin-streptomycin mixture. The cells were cultured and maintained in an incubator under conditions of 5% CO_2, 37°C, and appropriate humidity. The culture medium was replaced every two days.

2.2 Cytotoxicity test and morphological observation

Saffron extract (comprising 20% safflower oil and 80% sunflower seed oil) was prepared. HaCaT were seeded onto a 96-well plate and cultured in complete medium for 24 hours. Subsequently, the saffron extract was added, and the cells were incubated for an additional 24 hours. Following this, CCK-8 solution (purchased from Beyotime) was added, and the plate was incubated at 37°C for 1 hour. The absorbance at 450 nm was then measured using a microplate reader. Additionally, the morphological changes in the treated cells were observed under a microscope.

2.3 RNA Extraction and Quantitative Reverse Transcription (qRT-PCR)

Total RNA was extracted from HaCaT cells using TRIzol reagent. The concentration of the extracted RNA was measured using a NanoDrop UV-Vis spectrophotometer. Reverse transcription was carried out using the NovoScript®Plus All-in-one 1st Strand cDNA Synthesis SuperMix (with gDNA Purge). The relative mRNA expression level of 11β-HSD1 was determined using qRT-PCR with the NovoStart® SYBR qPCR SuperMix Plus on a LightCycler® Nano Instrument (from Roche Diagnostics). GAPDH was used as the reference gene for normalization, and the data were analyzed using the ΔΔCt method.

2.4 Statistical analysis

All statistical analysis was performed using SPSS software. Statistical significance was tested using analysis of variance with Tukey's test and Chi square test. $p < 0.05$ was considered statistically significant.

3. Results

Saffron extract, when tested at concentrations of 0.007813%, 0.015625%, 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, and 1%, did not significantly affect the viability of HaCaT cells, as demonstrated by the results presented in Table 1. Additionally, the morphological features of HaCaT cells remained unchanged when exposed to saffron extract at concentrations below 1%, as shown in Figure 1. By synthesizing the outcomes from the CCK-8 assay and cellular morphological observations, we can conclude that saffron extract exhibits no cytotoxicity towards HaCaT cells at concentrations up to 1%.

Saffron extract, at concentrations of 0.5% and 0.01%, was selected for the assessment of 11β-HSD1 gene expression. The results are presented in Table 2. Our findings indicate that saffron extract is capable of downregulating the expression of the 11β-HSD1 gene in HaCaT cells in a concentration-dependent manner. Specifically, treatment with 0.01% saffron extract resulted in a 35% reduction in 11β-HSD1 gene expression, whereas treatment with 0.5% saffron extract led to a 78% decrease in 11β-HSD1 gene expression.

Table 1. cell viability

	concentration							
	0.007813	0.015625	0.03125	0.0625	0.125	0.25	0.5	1
Mean	96.98%	96.20%	95.03%	95.85%	95.39%	95.35%	93.29 %	91.94 %
SD	3.03%	4.75%	1.52%	1.17%	2.11%	1.66%	2.42 %	4.93 %

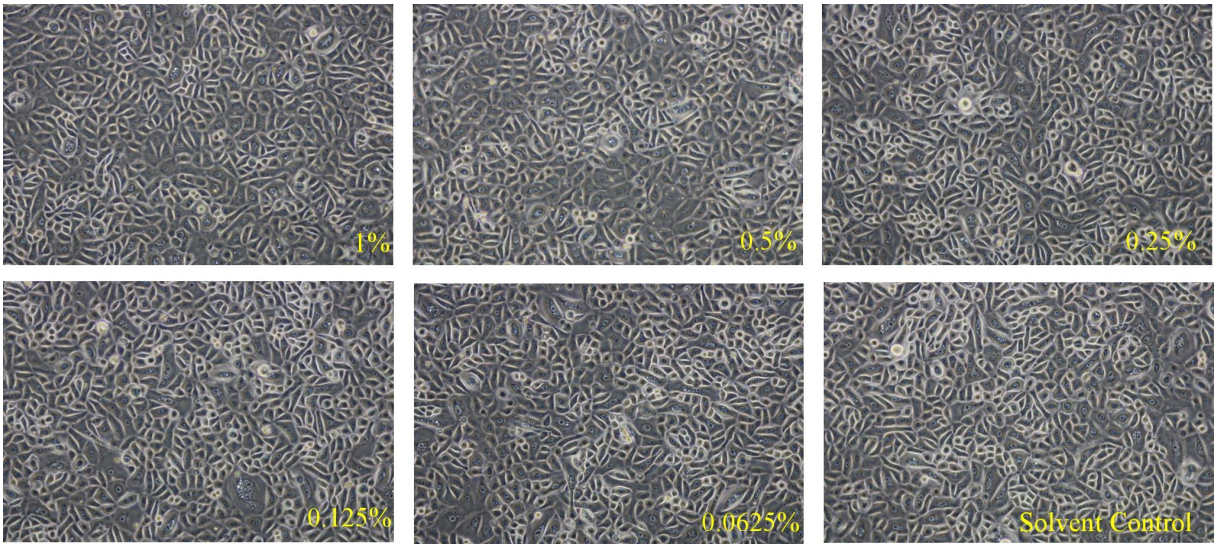

Figure 1. cell morphology

Table 2. 11β-HSD1 gene expression level

	Mean	SD	*P*-value	Down-regulation（*vs* BC）
BC	1	0.11	/	/
0.5%	0.22	0.02	0.000**	78%
0.01%	0.65	0.05	0.008**	35%

4. Discussion

Both cell viability assays and morphological observations have indicated that saffron extract exhibits no cytotoxic effects on HaCaT cells at concentrations up to 1%, suggesting its relative safety for use in cosmetic formulations. The enzyme 11β-HSD1 is present within the endoplasmic reticulum lumen of keratinocytes and is also expressed by dermal fibroblasts. This enzyme plays a crucial role in converting inactive cortisone into its active form, cortisol. Importantly, 11β-HSD1 is subject to positive feedback regulation by cortisol; elevated cortisol levels enhance the expression of 11β-HSD1, which in turn amplifies cortisol production, thereby exacerbating the deterioration of skin barrier function [21].Saffron extract has been demonstrated to downregulate the expression level of the 11β-HSD1 gene in HaCaT cells in a concentration-dependent manner. This finding suggests that saffron extract may modulate skin cortisol levels by influencing 11β-HSD1 expression, potentially alleviating the increase in skin cortisol induced by stress. Consequently, it could promote the proliferation of

keratinocytes and fibroblasts, maintain skin barrier integrity, and facilitate skin wound healing [22, 23].A notable side effect of glucocorticoid (GC) therapy is the induction of skin atrophy through the reduction of type I and III collagen expression. Experimental evidence has confirmed that subcutaneous administration of an 11β-HSD1 inhibitor can increase dermal thickness and collagen content [24]. By inhibiting 11β-HSD1 gene expression, saffron extract may possess potential anti-wrinkle properties.Furthermore, skin cortisol levels can reciprocally influence the skin HPA axis, which in turn regulates the central HPA axis, thereby mitigating the deleterious effects of stress on the skin [25]. However, the effects and mechanisms of topical saffron application on the central HPA axis require further experimental investigation.

5. Conclusion

This study validated through cytotoxicity assays and cellular morphological analysis that saffron extract exhibits no cytotoxic effects on HaCaT cells at concentrations of 1% or below. Furthermore, the extract significantly inhibits the expression of the 11β-HSD1 gene in HaCaT cells in a concentration-dependent manner, with the inhibition rate increasing proportionally with concentration. Specifically, at a concentration of 0.01%, saffron extract achieves a 35% reduction in 11β-HSD1 gene expression. Mechanistically, saffron extract may modulate skin cortisol metabolism by influencing 11β-HSD1 expression, thereby mitigating stress-induced increases in skin cortisol levels. This action contributes to the maintenance of skin barrier integrity, the promotion of keratinocyte and fibroblast proliferation, and the potential attenuation of skin aging processes. Collectively, these findings provide theoretical and experimental support for the development of saffron extract as an ingredient in mood-regulating skincare cosmetics. Future research directions include further exploration of the mechanisms underlying the topical application of saffron extract in modulating stress-related emotional responses.

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