



## ORIGINAL ARTICLE

### The effect of saffron serum on collagen density, inflammatory, and autophagy gene expression in UVB-exposed Wistar rats

Chitra Octavia<sup>1</sup>, Julia Windi Gunadi<sup>1,2</sup>, Oeij Anindita Adhika<sup>3</sup>, Lani Ishak<sup>1</sup>,  
Diana Krisanti Jasaputra<sup>1,4</sup>, Alexandrina Everdine Rosali<sup>1</sup>, and Ardo Sanjaya<sup>3</sup>

<sup>1</sup>Master Program of Skin Ageing and Aesthetic Medicine, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia

<sup>2</sup>Department of Physiology, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia

<sup>3</sup>Department of Anatomy, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia

<sup>4</sup>Department of Pharmacology, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia

 [julia.windi@maranatha.ac.id](mailto:julia.windi@maranatha.ac.id)

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#### ABSTRACT

##### BACKGROUND

Skin aging is a complex biological phenomenon influenced by intrinsic and extrinsic factors. Photoaging can be prevented by applying phytochemicals that have sun-protective properties. This study aimed to evaluate the effect of saffron serum to restore collagen density and autophagy processes and reduce inflammatory gene expression in UVB-exposed Wistar rats.

##### METHODS

An experimental laboratory study was conducted involving 20 male Wistar rats that were divided into 4 groups: control, UVB, UVB + base serum, UVB + saffron serum exposed to UVB radiation for 5 weeks with a total dose of 3100 mJ/cm<sup>2</sup>. The skin was extracted then underwent Masson Trichrome staining and real-time PCR to obtain collagen density and gene expression.

##### RESULTS

The gene expression of MMP1, IL6, TNF $\alpha$ , LC3, and p62 was significantly increased in the UVB group compared to the control group. Topical administration of saffron serum significantly increased collagen density ( $p=0.001$ ). Induction by UVB significantly increased LC3 ( $p=0.020$ ) and p62 ( $p=0.030$ ) gene expression, indicating an inhibition of autophagy. The saffron serum might modulate autophagy by increasing LC3, but not significant ( $p=0.495$ ) and significantly decreasing p62 gene expression ( $p=0.001$ ). As for MMP1, IL6, and TNF $\alpha$ , no significant decrease in gene expression was found in the UVB + saffron serum group compared to the UVB group.

##### CONCLUSION

Saffron serum increases collagen density and modulates autophagy in the skin of UVB-exposed Wistar rats. Inflammatory markers were increased after UVB induction, but no differences were found after saffron serum topical administration.

**Keywords:** UVB, collagen density, MMP1, IL6, TNF $\alpha$ , autophagy, saffron, rats

## INTRODUCTION

As the largest and outermost organ of the body, the skin has a major role in protecting against external environmental damage.<sup>(1)</sup> With three layers in its structure (epidermis, dermis, and hypodermis), the skin is a barrier to physical, chemical, neuronal, immune, and microbial stimuli.<sup>(2–4)</sup> Skin aging could be defined simply as skin changes caused by aging.<sup>(5)</sup> Based on epidemiological factors, skin aging could be divided into intrinsic (chronological and genetic) and extrinsic (environmental).<sup>(5–7)</sup> The environmental factors might include exposure to ultraviolet radiation (UVR) or sun exposure, air pollution, smoking, etc.<sup>(8–10)</sup> The skin aging process consists of morphological, histological, and physiological changes.<sup>(5)</sup> The changes considered characteristic of skin aging are degradation of collagen and elastic fibers in the dermis, epidermal thinning, and deleterious physiological changes in fibroblasts, resulting in skin wrinkles and reduced elasticity.<sup>(5,8,11)</sup> As for physiological changes, skin aging induces changes in barrier function, wound healing, endocrine and immune functions, even increasing the risk of skin cancer.<sup>(5,6,8)</sup>

The main environmental factor contributing to skin aging is UVR, a type of electromagnetic energy with detrimental as well as beneficial effects on the human skin, that is present in sunlight but can also be produced by artificial sources.<sup>(8)</sup> First discovered in 1801, UVR currently is divided by wavelength into UVA2 (320–340 nm), UVA1 (340–400 nm), UVB (280–320 nm), narrowband UVB (311–313 nm), and UVC (100–280 nm).<sup>(8,12)</sup> UVC is blocked by the earth's atmosphere, while UVA and UVB reach the surface of the earth and can penetrate the skin into the epidermis (UVB) and dermis (UVA).<sup>(8,12,13)</sup> The detrimental effects of UVR may be described in terms of molecular pathways, ranging from oxidative stress, DNA damage, cell cycle arrest and apoptosis, collagen degradation, dysregulated autophagy, inflammation, and immunosuppression.<sup>(6,8,11)</sup>

Ultraviolet irradiation of the skin (photoaging) induces reactive oxygen species (ROS) which act on the mitogen-activated protein kinase (MAPK) pathway, increasing activator protein 1 (AP-1), which leads to increased matrix metalloproteinases (MMPs).<sup>(6,8,11)</sup> These MMPs contribute to reduced skin elasticity, because they function as proteolytic enzymes that degrade extracellular matrix components such as

glycoproteins and collagen.<sup>(8,14)</sup> Matrix metalloproteinase-1 (MMP-1) is an enzyme that is mainly responsible for collagen degradation in the skin.<sup>(15)</sup> Production of reactive oxygen species also activates the nuclear factor kappa B (NF- $\kappa$ B) pathway, thus increasing pro-inflammatory cytokines such as interleukin 1 $\beta$  (IL1 $\beta$ ), interleukin 6 (IL6), and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ).<sup>(8)</sup> Autophagy, referring to macroautophagy, is a survival mechanism to protect the cells against apoptosis by recycling damaged cellular organelles and proteins to maintain intracellular homeostasis.<sup>(16)</sup> Some studies have proven an inhibition of autophagy after chronic exposure of fibroblasts to UVB radiation.<sup>(17,18)</sup> Microtubule-associated proteins 1A/1B light chain 3B (LC3) and sequestosome 1/SQSTM1 (p62) are autophagy markers commonly used to indicate activation of autophagy.<sup>(19)</sup>

Research about the prevention of photoaging using herbal-derived products is still ongoing and promising.<sup>(20,21)</sup> Known for its strong antioxidant properties, saffron (*Crocus sativus* L.) contains compounds such as crocin, crocetin, and safranal, which show potential anti-aging effects.<sup>(22,23)</sup> Previous studies have demonstrated that saffron may protect the skin against UV-induced damage. Improvement in wrinkle grade and increase in skin elasticity were found after application of saffron extract and avocado oil cream for 12 weeks on human subjects.<sup>(24)</sup> Crocin is a natural carotenoid antioxidant obtained from the stigmas of saffron flowers.<sup>(25)</sup> An in vitro study on crocin has proven its beneficial effects on human dermal fibroblasts irradiated by UVB, through decreasing ROS and increasing Col-1 and extracellular matrix production.<sup>(26)</sup> Research has shown that crocetin protects human skin fibroblasts against UVA damage by lowering ROS levels and minimizing cell apoptosis.<sup>(27)</sup> Madan and Nanda investigated the antiaging potential of safranal - the main compound in saffron- through dermal enzyme inhibition studies (activity against skin enzymes namely collagenase, elastase and hyaluronidase) along with determination of its sun protection factor (SPF).<sup>(28)</sup> Their results indicated significant inhibitory activity of safranal on MMPs responsible for aging and on SPF, establishing that this bioorganic molecule is a strong photoprotective agent.

While prior research has primarily focused on the in vitro effects of individual saffron compounds (e.g., crocin, crocetin, and safranal) or the clinical outcomes of saffron-based cosmetic

formulations, the mechanistic understanding of saffron's impact on skin at the molecular level remains underexplored. Most studies have examined endpoints such as ROS levels, enzyme inhibition, and collagen production, but have not comprehensively investigated changes in pro-inflammatory-related gene expression or autophagy-related markers *in vivo*. The present study introduces a novel approach by evaluating the effect of topical saffron serum (the term used in cosmetics for a concentrated emulsion) on specific molecular markers of skin aging and repair, especially pro-inflammatory (IL-6 and TNF- $\alpha$ ) and autophagy (LC3 and p62) gene expression, using an *in vivo* Wistar rat model exposed to UVB radiation. Therefore, this study aimed to examine the effect of topical saffron serum on collagen density, MMP-1, IL-6, TNF- $\alpha$ , LC3, and p62 gene expression in Wistar rats exposed to UVB rays.

## METHODS

### Research design

An experimental laboratory study was conducted in the Animal Laboratory of Universitas Kristen Maranatha, Bandung, Indonesia, from January 2024 until July 2024.

### Experimental animals

The subjects used in the study were 20 male Wistar rats aged 6-8 weeks, weighing 200-220 grams from Institut Teknologi Bandung, Bandung, Indonesia. The formula used in this study to determine minimum and maximum sample sizes per group was:  $n = (DF/k) + 1$ , where  $n$  represents the number of subjects per group,  $DF$  represents the error degrees of freedom (ranging from 10 to 20), and  $k$  represents the number of groups. Using the minimum  $DF$  of 10, the calculation yields  $n = (10/4) + 1 = 3.5$ , rounded to 4. For the maximum  $DF$  of 20, the calculation yields  $n = (20/4) + 1 = 6$ , rounded to 6. Therefore, the required sample size per group ranges from 4 to 6. The total sample size for the experiment was obtained by multiplying  $n$  by  $k$ , resulting in a range of 16 to 24 subjects across all groups. The total sample size used in the study was 20 male Wistar rats. This ensures that the error degrees of freedom remains within the acceptable range of 10 to 20.<sup>(29)</sup> Upon arrival, the rats underwent environmental adaptation for 1 week. They were kept in a laboratory with a 12-hour light and dark cycle, given standard chow and water *ad libitum*. Then the rats were divided into 4 groups: control,

UVB, UVB + base serum, and UVB + saffron serum. The control group did not receive any treatment, UVB group was exposed to UVB radiation without any topical application, UVB + base serum group was exposed to UVB radiation and base serum topical application, UVB + saffron group was exposed to UVB radiation and saffron serum topical application.

### Preparation of saffron serum

The saffron serum is produced by PT. Lipwih Synergylab Estetika, a cosmetics manufacturing company. The saffron used in the formulation is imported from Afghanistan, a region renowned for its high-quality saffron with vibrant red threads (stigmas) and strong aromatic properties. To prepare a serum containing 8% saffron extract, 8 grams of saffron extract were mixed with 92 grams of distilled water and left to steep for 24 hours. The mixture was then filtered to obtain the liquid extract, which was used as the active ingredient. For the gel phase, 70 grams of distilled water were heated to 70–75°C, and hydroxy ethyl cellulose (HEC) was gradually added while stirring continuously. A homogenizer was used to ensure a smooth and uniform mixture, which was then cooled to 40°C.

At 40°C, the saffron extract was added to the gel phase and homogenized thoroughly until the serum reached a uniform consistency. A small sample was taken for quality control (QC) analysis, and after confirming satisfactory results, the total weight of the serum was measured. The production yield was calculated by dividing the final weight of the serum by the total initial weight of ingredients and multiplying by 100%. The finished serum was then stored in labeled containers in a designated storage area.

### UVB radiation, saffron serum, and sample collection

The UVB exposure was carried out by the investigators and animal lab staff using a narrowband UVB lamp with a maximal intensity at 311 nm. The rats were exposed to UVB radiation and saffron serum for 5 weeks. The total dose of UVB was 3100 mJ/cm<sup>2</sup>, while the topical serum concentration was 8% with total volume of 0.1 mL/cm<sup>2</sup>, applied 20 minutes before and after application of UVB radiation for three days per week. The radiated skin area of 4x4 cm was on the dorsal surface of the rat skin, and made hairless by shaving once a week with a razor during the study. One day after the last UVB radiation, the rats were

terminated, and then the skin tissue was collected for Masson trichrome staining and real-time PCR.

### Collagen density measurement

The skin tissues from the terminated rats were made into paraffin blocks, and cut into 5  $\mu$ m sections, then Masson trichrome staining was used to measure collagen density. Images for each slide were taken using the CX21 Olympus Microscope at 100x magnification, with 24-bit RGB resolution. The collagen density was measured using Image J software from 5 slides for each subject. The Region of Interest (ROI) on the slide was first selected and then deconvoluted into 2 monochromatic 8-bit images, with blue representing collagen and red representing muscle. The procedure was continued with auto thresholding and then quantitative analysis to obtain mean, minimal, and maximal intensities, and standard deviation. The collagen density was obtained from the mean intensity in arbitrary units (AU).<sup>(30)</sup>

### Realtime PCR

RNA extraction from the rat skin was done using Genezol (GZR200, Geneaid Biotech Ltd., New Taipei City, Taiwan), following the manufacturer's instructions. Concentration and purity were then measured with Multiskan GO (Thermo Fisher Scientific, Vantaa, Finland). Quantitative PCR was conducted using a SensiFAST SYBR No-ROX One-Step RT-PCR Kit (BIO-72005, Bioline, London, United Kingdom), according to the manufacturer's

instructions. Primer sequences used in this study are shown in Table 1.

### Ethical clearance

All animal protocols of the experiment were approved by the Research Ethics Committee of Universitas Kristen Maranatha Bandung, under Number 011/KEP/II/2024.

### Statistical analysis

The statistical analysis was done using SPSS version 26 and the data were presented as means  $\pm$  SEM (Standard Error of the Mean), with a significance level set at 0.05. One Way ANOVA was used to compare the differences between groups, and the LSD post hoc test was used to indicate which groups were different from the other groups.

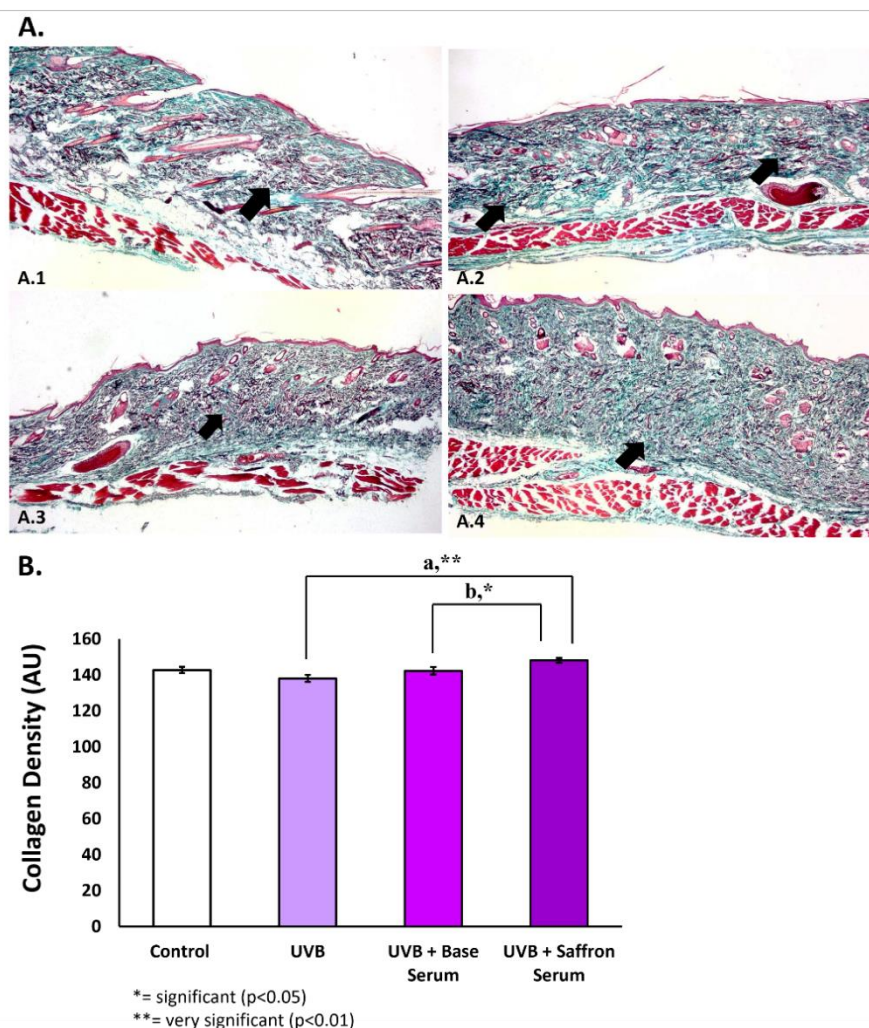
## RESULTS

### The effect of saffron serum on collagen density

The result of Masson trichrome staining is presented in Figure 1A. In this study, the mean collagen density  $\pm$  SEM results in arbitrary units were as follows: Control  $142.75 \pm 1.75$  AU; UVB  $138.09 \pm 1.94$  AU; UVB + base serum  $142.31 \pm 2.03$  AU; UVB + saffron serum  $148.09 \pm 1.36$  AU. One Way ANOVA test result showed  $p=0.01$ , while the LSD post hoc test only showed a significant difference between UVB and UVB + saffron serum groups ( $p=0.001$ ) and between UVB + base serum and UVB + saffron serum ( $p=0.036$ ). The results are shown in Figure 1B.

**Table 1.** Primer Sequences Used for the Realtime PCR

| Gene Symbol  | Primer Sequence (5' to 3')                         | Product size (bp) |
|--------------|----------------------------------------------------|-------------------|
|              | Upper strand: sense<br>Lower strand: antisense     |                   |
| MMP1         | GGCAAATGCAGCAGTTATTTGGGC<br>ATGGGGCCACATCAGGCACCC  | 105 bp            |
| IL6          | GAAGTTAGAGTCACAGAAGGAGTG<br>GTTTGCCGAGTAGACCTCATAG | 105 bp            |
| TNF $\alpha$ | GTCGTAGCAAACCACCAAGC<br>TGTGGGTGAGGAGCACATAG       | 187 bp            |
| LC3          | GGTCCAGTTGTGCCTTTATTG<br>GTGTGTGGGTTGTGTACGTCG     | 153 bp            |
| p62          | CTAGGCATCGAGGTTGACATT<br>CTTGGCTGAGTACCACTCTTATC   | 116 bp            |
| GAPDH        | GTTACCAGGGCTGCCTTCTC<br>GATGGTGATGGGTTTCCCGT       | 177 bp            |



**Figure 1.** Collagen deposition and collagen density after topical administration of saffron serum: A. Masson trichrome staining (black arrow = collagen deposition); B. Collagen density (UVB and UVB + saffron serum group ( $p=0.001$ ) (a) and between UVB + base serum and UVB + saffron serum ( $p=0.036$ ) (b) in arbitrary units (AU)

### The effect of saffron serum on MMP1 gene expression

As for MMP1 gene expression, the mean fold change in expression  $\pm$  SEM was as follows: Control  $1.00 \pm 0.17$ ; UVB  $2.08 \pm 0.60$ ; UVB + base serum  $2.87 \pm 0.42$ ; UVB + saffron serum  $2.74 \pm 0.30$ . One Way ANOVA test result showed  $p=0.015$ , while the LSD post hoc test showed a significant difference between the control and UVB ( $p=0.040$ ), control and UVB + base serum ( $p=0.004$ ), and between the control and UVB + saffron serum ( $p=0.007$ ). The results are shown in Figure 2A.

### The effect of saffron serum on IL6 and TNF $\alpha$ gene expression

In this study, the mean fold change in expression  $\pm$  SEM for IL6 gene expression was as follows: Control  $1.00 \pm 0.19$ ; UVB  $2.91 \pm 0.71$ ;

UVB + base serum  $2.20 \pm 0.59$ ; UVB + saffron serum  $3.07 \pm 0.34$ . One Way ANOVA test result showed  $p=0.029$ , while the LSD post hoc test showed a significant difference between the control and UVB ( $p=0.009$ ), and between the control and UVB + saffron serum ( $p=0.010$ ). The results are shown in Figure 2B.

As for TNF $\alpha$  gene expression, the mean fold change in expression  $\pm$  SEM was as follows: Control  $1.00 \pm 0.38$ ; UVB  $2.89 \pm 0.48$ ; UVB + base serum  $2.67 \pm 0.45$ ; UVB + saffron serum  $2.80 \pm 0.48$ . One Way ANOVA test result showed  $p=0.030$ , while the LSD post hoc test showed a significant difference between the control and UVB ( $p=0.011$ ), control and UVB + base serum ( $p=0.019$ ), and between the control and UVB + saffron serum ( $p=0.012$ ). The results are shown in Figure 2B.

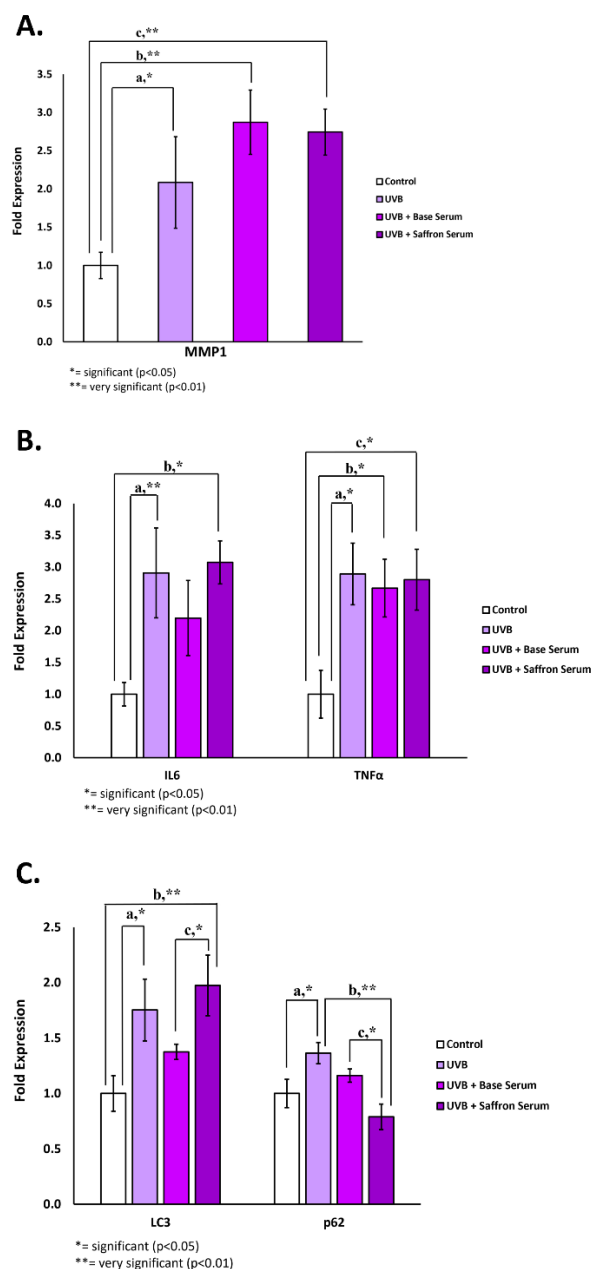


Figure 2. MMP1, inflammatory, autophagy gene expression after topical administration of saffron serum: A. MMP1 (control and UVB ( $p=0.040$ ) (a), control and UVB + base serum group ( $p=0.004$ ) (b), and between the control and UVB + saffron serum ( $p=0.007$ ) (c)); B. IL6 (control and UVB ( $p=0.009$ ) (a), and between control and UVB + saffron serum ( $p=0.010$ ) (b)); TNFα (control and UVB ( $p=0.011$ ) (a), control and UVB + base serum group ( $p=0.019$ ) (b), and (control and UVB + saffron serum ( $p=0.012$ ) (c); C. LC3 (control and UVB ( $p=0.020$ ) (a), control and UVB + saffron serum ( $p=0.005$ ), UVB and UVB + saffron serum ( $p=0.495$ ) (b), between UVB + base serum and UVB + saffron serum ( $p=0.045$ ) (c), p62 (control and UVB ( $p=0.030$ ) (a), UVB and UVB + saffron serum group ( $p=0.001$ ) (b), and between the UVB + base serum and UVB + saffron serum ( $p=0.029$ ) (c)

## The effect of saffron serum on LC3 and p62 gene expression

In this study, the mean fold change in expression  $\pm$  SEM for LC3 gene expression was as follows: Control  $1.00 \pm 0.16$ ; UVB  $1.75 \pm 0.28$ ; UVB + base serum  $1.38 \pm 0.07$ ; UVB + saffron serum  $1.98 \pm 0.27$ . One Way ANOVA test result showed  $p=0.020$ , while the LSD post hoc test showed a significant difference between the control and UVB ( $p=0.020$ ), and between the control and UVB + saffron serum ( $p=0.005$ ), between UVB + base serum and UVB + saffron serum ( $p=0.045$ ). The result is shown in Figure 2C. LC3 gene expression in UVB + saffron serum has the highest mean, but a non-significant difference was found between UVB group and UVB + saffron serum group ( $p=0.495$ ).

As for p62 gene expression, the mean fold change in expression  $\pm$  SEM was as follows: Control  $1.00 \pm 0.13$ ; UVB  $1.36 \pm 0.10$ ; UVB + base serum  $1.16 \pm 0.06$ ; UVB + saffron serum  $0.79 \pm 0.12$ . One Way ANOVA test result showed  $p=0.011$ , while the LSD post hoc test showed a significant difference between the control and UVB ( $p=0.030$ ), between UVB and UVB + saffron serum ( $p=0.001$ ), and between the UVB + base serum and UVB + saffron serum ( $p=0.029$ ).

## DISCUSSION

In this study, the collagen density was increased in the group that received topical administration of saffron serum compared to UVB and UVB + base serum groups (Figure 1). This study showed that UVB induction significantly increased MMP1 gene expression in all UVB groups compared to control, while no difference was found between UVB + saffron serum compared to the UVB group alone (Figure 2). We also found a significant increase in IL6 and TNFα gene expression after induction of UVB radiation, although we did not find any difference between the group treated with saffron serum compared to other groups. Interestingly, we found an increase in LC3 and p62 gene expression in UVB group, suggesting an inhibition of autophagy, while saffron serum topical administration increased LC3 and decreased p62 gene expression, suggesting an activation of autophagy.

The finding of increased collagen density is supported by another study which stated that saffron was able to neutralize UVA and UVB-induced oxidative stress in a human keratinocyte cell line (HaCat cells).<sup>(31)</sup> Research by Xiong et al.<sup>(32)</sup> using human dermal fibroblasts (HDFs),

proved the saffron extract's capability to promote collagen and hyaluronic acid synthesis in wound healing.

The results of MMP1 gene expression are contradictory to the research conducted by Xiong et al.<sup>(32)</sup> which found the inhibition activities of saffron on collagenases. Nevertheless, MMP1 is not the only collagenase with the function of degrading skin collagen, because there are other collagenases such as MMP3, MMP8, MMP9, MMP13, and MMP18 with the same function.<sup>(15)</sup> Some studies found that saffron inhibited MMP9 in arthritis and multiple sclerosis,<sup>(33–35)</sup> while another study found that saffron intake attenuated MMP-13 in chondrocytes and MMP-3 in human epithelial cells after induction of inflammation.<sup>(36)</sup> The decomposition of dermal collagen type I and III is a combined action of MMP1, MMP3, and MMP9, giving rise to products that finally decrease collagen synthesis.<sup>(6)</sup>

The results of this study (IL6 and TNF $\alpha$  gene expression) are contradictory to a study by Xiao et al.<sup>(37)</sup> which found a significantly decreased TNF $\alpha$  in the brain of 8- and 16-month-old mice after being given saffron extract orally for 8 weeks. Another study on 64 T2DM patients who received either 15 mg of saffron or placebo capsules (two pills per day) for 3 months, found that interleukin-6 (IL-6), and tumor necrosis factor (TNF- $\alpha$ ) increased significantly in both groups ( $p < 0.05$ ).<sup>(38)</sup> In contrast, a randomized, double-blind, placebo-controlled trial involving 66 women older than 18 years with rheumatoid arthritis, who received 100 mg/day either saffron supplement or placebo for 12 weeks, found non-significant decreases in tumor necrosis alpha and interferon gamma.<sup>(39)</sup> Another study stated that the magnitude of proinflammatory cytokine reduction varies depending on the doses.<sup>(40)</sup> Crocin and its derivate crocetin, components of saffron, have shown protective effects against UVA or UVB radiation by decreasing intracellular ROS and inflammatory molecules, as well as modulating the NF- $\kappa$ B pathway.<sup>(26,27,41)</sup> Another component of saffron, namely safranal, was studied for its sun-protective and moisturizing effects at a concentration of 8%. The study concluded that 8% of nanoliposome safranal has better sun protective effects compared to the homosalate reference.<sup>(42)</sup>

The results of autophagy gene expression (LC3 and p62) are in line with some studies, where crocin and crocetin from saffron were found to modulate autophagy in cutaneous squamous cell carcinoma, colorectal carcinoma, and cervical cancer.<sup>(43–45)</sup> However, the modulation of

autophagy by saffron extract in animals with induced photoaging needs further study.

The skin is the largest organ in the human body, responsible for protecting the body from various environmental factors, including ultraviolet (UV) radiation.<sup>(46)</sup> UV radiation, especially UVB, is one of the main factors that contribute to skin aging.<sup>(8,13)</sup> UVB radiation penetrates the skin and induces the formation of reactive oxygen species (ROS), which can lead to DNA damage, inflammation, and the activation of matrix metalloproteinases (MMPs), particularly MMP-1.<sup>(6,8,15)</sup> MMP-1 is an enzyme that breaks down collagen in the dermis, leading to a loss of skin elasticity and the formation of wrinkles, which are common signs of skin aging.<sup>(15)</sup> Furthermore, ROS also activates mitogen-activated protein kinase (MAPK) which then induces activator protein-1 (AP-1), blocking Smad2/3 which regulates transforming growth factor beta (TGF $\beta$ ), thus inhibiting collagen synthesis which eventually decreases collagen density.<sup>(6)</sup> Saffron has been widely recognized for its antioxidant properties, attributed to its bioactive components such as crocin, crocetin, and safranal.<sup>(23)</sup> These compounds have been shown to neutralize ROS, reduce inflammation, and prevent collagen degradation.<sup>(22,47–49)</sup>

UVB radiation also induces nuclear factor- $\kappa$ B (NF- $\kappa$ B), activating proinflammatory cytokines, such as IL6, IL1, and TNF $\alpha$  that lead to increases in collagen and elastin degradation, resulting in premature aging skin.<sup>(50,51)</sup> The anti-inflammatory action of saffron serum has been studied in arthritis, diabetes, and sub-chronic stress in humans and animals, where saffron extracts were given through oral or injection routes.<sup>(39,52–54)</sup>

As a key regulator of homeostasis and disease, autophagy is a survival mechanism by which the damaged organelle undergoes lysosomal degradation and recycling to maintain homeostasis.<sup>(55)</sup> The autophagy process begins with the formation of a double-membrane autophagosome, fusion to the lysosome, and continues with the degradation of its contents.<sup>(55)</sup> When autophagy is activated, LC3 lipidation causes its moving into the autophagosome, while sequestosome 1 (p62/SQSTM1), a classical selective autophagy receptor, localizes with ubiquitinated protein aggregates or is degraded in a lysosome-dependent manner in macroautophagy.<sup>(19)</sup>

Based on the findings of this study, the topical administration of saffron serum might exhibit potential in mitigating UVB-induced

photoaging through its impact on collagen density and autophagy modulation, despite its limited effects on pro-inflammatory cytokines and MMP-1 expression. The non-significant increase in LC3 and the significant decrease in p62 gene expression suggest that saffron serum may play a role in restoring autophagic processes disrupted by UVB exposure, offering a novel mechanism of action for its photoprotective properties. While the study highlights the antioxidant and potential autophagy-modulating effects of saffron components, the contradictory findings regarding MMP-1 and cytokine expression emphasize the complexity of saffron's biological effects, which may vary depending on factors such as dose, mode of application, and experimental conditions. Future research should focus on identifying the specific molecular targets of saffron components, particularly their effects on different collagenases and pro-inflammatory pathways, to further elucidate their therapeutic potential in managing UVB-induced photoaging and other skin disorders.

The limitations of the study are: (1) the study did not measure anti-inflammatory cytokines such as IL4 and IL10, other MMPs such as MMP3, MMP9, and MMP13, and other markers of autophagy such as ATG5 and Beclin; (2) saffron was given in the form of a concentrated serum, not in the form of nanoliposomes; (3) the duration of the study should have been longer to obtain the more sustainable effects of saffron extract on rat skin with induced photoaging.

Despite these limitations, this study provides valuable insights into the potential of saffron as a topical agent for preventing skin aging. Future studies with different forms of saffron extract, longer treatment durations, and measurements of other anti-inflammatory cytokines, MMPs, and autophagy markers will strengthen saffron's potential against skin aging.

## CONCLUSIONS

Saffron extract has some crucial compounds that might be beneficial as anti-inflammatory and anti-collagenase agents, and maintaining homeostasis through autophagy in UVB-induced skin. Topical administration of saffron extract increases collagen density and modulates autophagy in the skin induced by UVB radiation, mimicking the external aging process. Further study needs to confirm the effects of saffron extract on broader anti-inflammatory, collagenase,

elastase, and other autophagy markers, to ascertain the potential of saffron in alleviating skin aging.

## Conflict of Interest

Competing interests: No relevant disclosures.

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## Author Contributions

CO, OAA, and JWG provided the initial idea for the research; CO, LI, and AER conducted the animal experiment, histopathology, and real-time PCR; DKJ and AS analyzed and interpreted the data and supervised the experiment; CO, JWG, and OAA drafted the manuscript. All authors have read and approved the final manuscript.

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## Data Availability Statement

Original data used in this study could be requested directly from the corresponding author.

## Declaration of Use of AI in Scientific Writing

The authors used Grammarly to improve English writing, and then all authors reviewed and edited the content before submitting the manuscript.

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