Effect of turmeric (*Curcuma longa*) extract administration on tumor necrosis factor alpha and type 1 collagen expression in UVB-light radiated BALB/c mice

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ABSTRACT

Skin aging caused by chronic exposure to ultraviolet is called photoaging. Ultraviolet B (UVB) is also known to cause skin cancer. On the other hand, curcumin, an active component found in turmeric (*Curcuma longa*), is a potent antioxidant. Herein, with rising skin cancer rates and interest in preventing photoaging, this study aims to analyze the photoprotective potential of topical turmeric extract with pharmacological activity against UVB. This experiment used 32 BALB/c mice, and the mice were randomly divided into 4 groups: normal control group (NC), sick control/ UVB-exposed group (SC), UVB-exposed + vehicle group (VT), and UVB exposed + topical turmeric extract group (TT). Except for group NC, all groups received UVB radiation 6 times/week for 4 weeks (24 times in total). At the end of the treatment, the skin tissues were examined using immunohistochemistry. From the interpretation of immunohistochemistry results, there was a significant decrease in tumor necrosis factor expression and an increase in type 1 collagen for TT compared to the control ($p < 0.05$). In conclusion, the *C. longa* might have a potential photoprotector property in preventing photoaging.

INTRODUCTION

Photoaging is premature skin aging caused by chronic sunlight exposure and characterized by rough wrinkles, rough texture, and loss of elasticity in the skin (Cavinato and Jansen-Dürr, 2017; Le Digabel *et al.*, 2018). UV is also reported to be responsible for 80% of facial skin aging (Zhang and Duan, 2018). In addition to photoaging, DNA damage, caused by short-wavelength ultraviolet B (UVB) exposure, can be the forerunner of skin cancer (Garg *et al.*, 2017; Pandel *et al.*, 2013). UVB that reaches the skin excites the skin chromophore and stimulates the production of reactive oxygen species (ROS). Namely, ROS will then activate factors such as the transcription factors nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1). The transcription factor NF-κB will transcribe proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1. However, the transcription factor AP-1 will transcribe matrix metalloproteinases (MMPs), which play an important role in collagen degradation, whereby it is deemed a hallmark of photoaging (Cavinato *et al.*, 2017; Garg *et al.*, 2017; Panich *et al.*, 2016; Rittié and Fisher, 2015; Sanches Silveira and Myaki Pedroso, 2014; Young *et al.*, 2017). Thus, for skin that is exposed to UVB, TNF-α expression will increase; however, type 1 collagen expression will decrease (Poon *et al.*, 2015; Zhi *et al.*, 2019).

Nowadays, the use of herbal products in preventing photoaging has increased substantially inasmuch as plant extracts are considered natural and safe skincare products. In this context, turmeric (*Curcuma longa*) is a rhizomatous herb that is cultivated and used as a kitchen spice in tropical regions such as India, China, Indonesia, Thailand, and Africa (Gopinath and Karthikeyan, 2018). A substance called curcumin in turmeric is known to have anti-inflammatory properties (Dunaway *et al.*, 2018).

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The topical turmeric extracted in this study was applied immediately after UVB exposure. Thus, the topical turmeric extract is presumed to act as an adjuvant for sunscreens and a compound that shields the skin from the photoaging UVA rays besides being for therapy. The benefits of turmeric as a photoprotector in various dosage forms have been investigated by several previous studies. These studies report that turmeric is a potent ingredient used as a photoprotector, especially when administered topically (Li *et al.*, 2016; Phillips *et al.*, 2013; Sumiyoshi and Kimura, 2009). Surprisingly, although there has been a lot of research about the effect of turmeric on the skin, the effect of a single long-term application of turmeric on collagen expression in skin exposed to UVB is still scant. Hence, this study aimed to examine the role of turmeric extract in preventing photoaging by analyzing the expression of TNF-α and type 1 collagen in BALB/c mice exposed to UVB (*in vivo*).

MATERIALS AND METHODS

Research design

This research was pure experimental research with a posttest-only control group design conducted in the Faculty of Pharmacy, Universitas Airlangga, Indonesia (*Ethical Clearance Certificate Number:* 447/HRECC.FODM/X/2020). Thirty-two mice were randomly divided into 2 control groups and 2 different treatment groups: normal control group (NC), sick control/UVB-exposed group (SC), UVB-exposed + vehicle group (VT), and UVB-exposed + topical turmeric extract group (TT). Groups SC, VT, and TT were exposed to UVB 24 times for 4 weeks (6 times/week). Still, immediately after exposure, groups VT and TT received topical vehicle (Vaseline) and 16% turmeric extract, respectively. Thereafter, mice were then evaluated on day 27 after treatment.

UVB exposure

The radiation process for mice was carried out by exposing mice to UVB light from a UVB lamp (Philips PL-S 9W/01/2P), which had been installed in the mice irradiation box. Before radiation, the dorsum area of the mice was shaved using a shaver to eliminate any resistance that blocks UVB from reaching the skin's surface. The dorsum of the mice was shaved with an area of 2.5×4 cm^2. Thereafter, the mice were exposed to a narrow spectrum of UVB (305–315 nm, peak 311 nm) at a dose of 1 minimal erythemal dose (MED), where 1 MED was 240 mJ/cm^2 (obtained from a preliminary study), at a distance of 18 cm above the dorsal mice, six times per week for 4 weeks, with an exposure duration of 35 minutes/exposure. The UVB radiation exposure was repeated 24 times with a total exposure dose of 5,760 mJ/cm^2.

Ointment application to mice

The turmeric extract ointment was only applied to the group of mice TT. Immediately, the vehicle and the turmeric extract were applied after UVB irradiation six times a week for 4 weeks (Agrawal and Kaur, 2010). On the mice's skin with an area of 2.5×4 cm^2, 1 g (two fingertip units) of turmeric extract ointment was applied (Oakley, 2016).

Skin tissue sampling

The skin tissue of the mice was taken on the 27th day after all treatments were completed. The mice were anesthetized

using ketamine at a dose of 50 mg/kg. Posteriorly, the dorsal skin tissue of the mice that had been exposed to UVB was excised to the depth of the dermis. After skin sampling, the mice were executed by cervical dislocation and buried. All skin tissue that had been obtained was then stored in a 10% formalin solution (formaldehyde 37%–40%). Subsequently, for examination, they were embedded in paraffin (wax) to create paraffin-embedded tissue blocks from TNF-α expression and type 1 collagen. The paraffin blocks were created using the immunohistochemical method at the Anatomical Pathology Laboratory, Faculty of Medicine, Airlangga University.

Immunohistochemistry

Immunohistochemical staining was performed using an anti-TNF-α monoclonal antibody [TNF-α (52B83): sc-52746, Santa Cruz Biotechnology, Inc.] and type 1 anti-collagen monoclonal antibody [COL1A1 (3G3): sc-293182, Santa Cruz Biotechnology, Inc.]. TNF-α expression was measured by counting the number of macrophage cells in the dermis that expressed TNF-α using a light microscope with 400× magnification in five fields of view. Type 1 collagen expression was measured by measuring the thickness of the collagen layer in the dermis (in micrometers) in five fields of view.

Statistical analysis

The research data obtained were then processed and statistically examined using Statistical Package for the Social Sciences for Windows Release 25.0. The statistical test chosen was the one-way analysis of variance (ANOVA) test and continued with the *post hoc* test [least significant difference (LSD)] to determine the difference between the undertaken treatments.
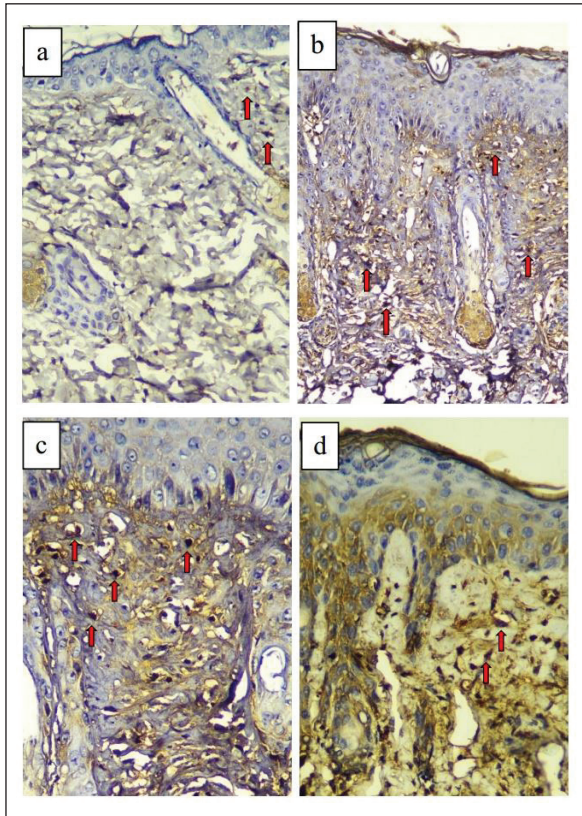
RESULTS AND DISCUSSION

Immunohistochemistry of TNF-α

The results demonstrated that the lowest expression of TNF-α was found in NC. Nevertheless, the highest expression was found in the group that was exposed to UVB and given the vehicle (VT). The results of TNF-α expression from the lowest to the highest, respectively, were the groups including NC, TT, SC, and VT (Figs. 1 and 2).

Additionally, the results of the one-way ANOVA test eliminated a significant value of $p < 0.001$. Hence, it can be concluded that there was a significant difference in the mean TNF-α expression between the treatment groups ($p < 0.05$). Furthermore, based on the results of the *post hoc* LSD test, it was found that there was a significant difference in almost all groups, including the groups NC and SC ($p = 0.000$), NC and VT ($p = 0.00000$), NC and TT ($p = 0.000$), and SC and TT ($p = 0.000$). However, contingent on the SC and VT groups, the results were not significantly different ($p = 0.238$).

Accordingly, it was derived that there was a significant difference between the means of TNF-α expression in SC and the NC ($p < 0$). Fortunately, it was found that all the findings coincided with a previous report that addressed that synergistic upregulation in TNF-α is counted as a key early response to UVB exposure (Bashir *et al.*, 2009a, 2009b). Bashir *et al.* (2009a, 2009b) showed that UVB exposure induced an increase in TNF-α expression mainly in epidermal keratinocytes. However, in this study, TNF-α expression could only be read and analyzed in macrophages.

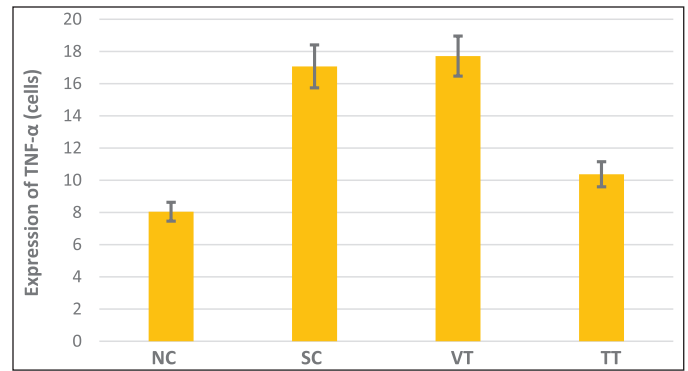**Figure 1.** Dorsal skin tissue of BALB/c mice stained with TNF-α immunohistochemistry (400× magnification). (a) Normal control group (NC). (b) Sick control/UVB-exposed group (SC). (c) UVB-exposed + vehicle group (VT). (d) UVB-exposed + topical turmeric extract group (TT). Macrophage cells that express TNF-α are stained brown (red arrows), while cells that do not express TNF-α are stained blue/purple.

Figure 2. Graph of TNF-α expression in all groups.

TNF-α receptor binding site through covalent and noncovalent interactions, thereby blocking the TNF-α-dependent activation of NF-κB (Vollono *et al.*, 2019).
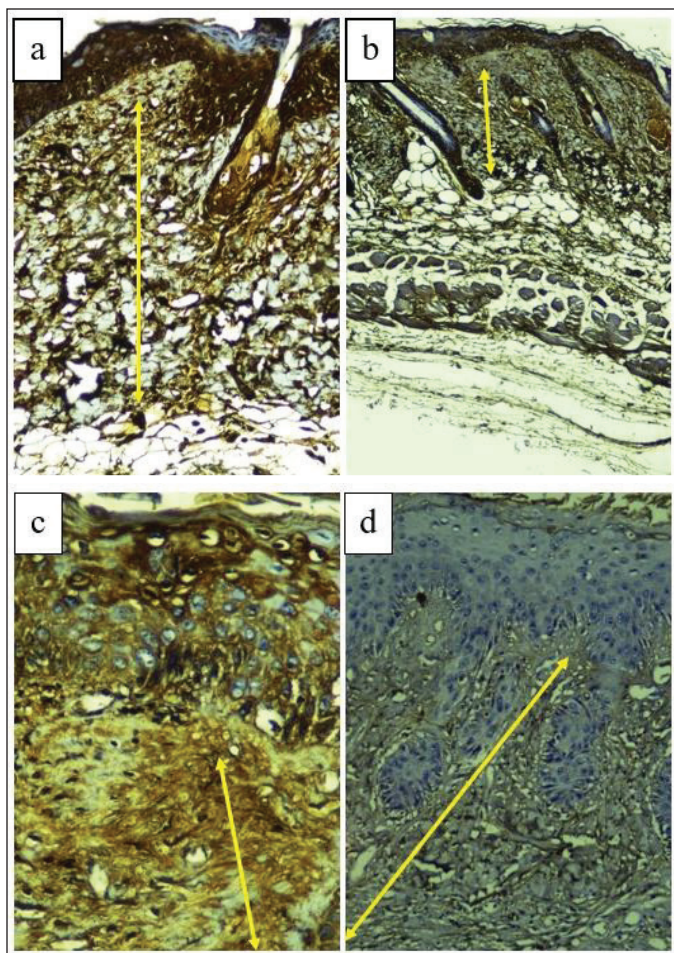
Immunochemistry of type 1 collagen

The results showed that the highest expression of type 1 collagen was found in the group exposed to UVB and given 16% turmeric extract ointment (TT), while the lowest expression was found in the group that was exposed to UVB only (SC). The results of the highest to lowest type 1 collagen expression, respectively, were TT, NC, VT, and SC (Figs. 3 and 4).

The results of the one-way ANOVA test showed a significant value of $p < 0.001$; hence, it can be concluded that there is a significant difference in the mean type 1 collagen expression between the treatment groups ($p < 0.05$). Furthermore, based on the results of the *post hoc* LSD test, it was found that there was a significant difference in the mean in groups NC and SC ($p < 0.001$), groups NC and VT ($p = 0.017$), groups SC and VT ($p = 0.007$), and groups SC and TT ($p < 0.001$). Meanwhile, the mean difference which was not significant was only found in groups NC and TT ($p = 0.443$).

In the results of the study above, the lowest thickness of type 1 collagen expression was found in SC with a significant difference in average compared to the NC ($p < 0.05$). These results are in line with the photoaging theory that states that long-term UVB exposure can cause degradation or a decrease in the amount of type 1 collagen. The photoaging process begins with the formation of ROS due to UVB exposure. Increased ROS will activate the MAPK pathway in macrophage cells and fibroblast cells. Activation of the MAPK pathway will induce the transcription factor AP-1, resulting in MMP-1 transcription. It is this MMP-1 that acts as the main enzyme for degrading type 1 collagen (Pandel *et al.*, 2013; Pittayapruek *et al.*, 2016).

In addition to inducing the degradation of type 1 collagen, UVB light can also reduce the expression of type 1 collagen by inhibiting its synthesis. ROS as a result of UVB exposure can cause a decrease in the transforming growth factor-β (TGF-β) receptor type 2, which is a receptor required by TGF-β to induce procollagen synthesis through the SMAD signaling pathway. ROS has also been reported to inhibit Smad2/Smad3 phosphorylation and nuclear translocation in procollagen synthesis (Ansary *et al.*, 2021; Mcdaniel *et al.*, 2018; Pittayapruek *et al.*, 2016). UVB-

Besides, it was assumed that the unclear TNF-α expression in epidermal keratinocytes was likely caused by the peroxidation reaction of immunohistochemical staining.

Furthermore, Bashir *et al.* (2009a, 2009b) reported that UVB exposure to the skin induces the formation of ROS. This increase in ROS can then cause damage to cell structures due to oxidative stress. Nucleic acids, lipids, and proteins that are modified by ROS or UVB exposure act as damage-associated molecular patterns that can activate sensors/receptors such as toll-like receptor (TLR) or cyclic guanosine monophosphate-adenosine monophosphate synthase on cell membranes and cytosols resulting in a signaling cascade that induces the transcription of proinflammatory cytokines such as TNF-α (Gallo and Bernard, 2014).

The results also showed that there was a significant difference in the mean TNF-α expression in TT compared to SC ($p < 0.05$). These results were in line with a previous report that stated that curcumin in turmeric is a strong intracellular antioxidant (Barzegar and Moosavi-Movahedi, 2011). Curcumin is also known to inhibit TNF-α promoters by methylation and can also inhibit TLR 2 and 4 signaling by lipopolysaccharide, which is responsible for TNF-α induction. In addition, curcumin can also directly bind to the

Figure 3. Dorsal skin tissue of BALB/c mice stained with type 1 collagen immunohistochemistry (400× magnification). (a) Normal control group (NC). (b) Sick control/UVB-exposed group (SC). (c) UVB-exposed + vehicle group (VT). (d) UVB-exposed + topical turmeric extract group (TT). Type 1 collagen expression was measured by measuring the thickness of the collagen layer in the dermis (μm) indicated by the yellow arrow.
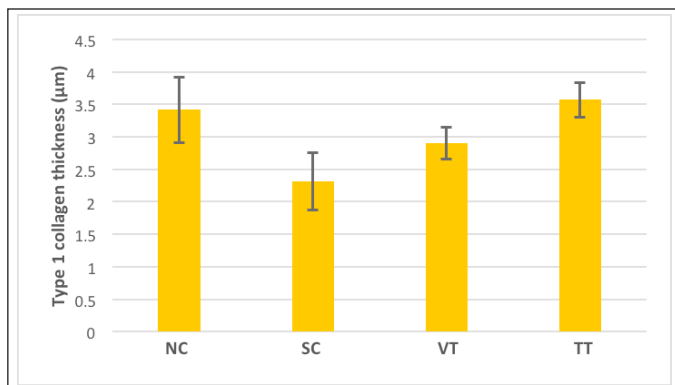
Figure 4. Graph of type 1 collagen expression in all groups.

induced ROS can also directly reduce the expression of TGF-β (Yin *et al.*, 2015). Therefore, UVB exposure can decrease the expression of type 1 collagen by inducing its degradation and inhibiting its synthesis. A decrease in the amount of type 1 collagen is a hallmark of external aging due to UV exposure (photoaging) (Poon *et al.*, 2015).

The mean expression of type 1 collagen in VT was higher than in SC ($p < 0.05$). This indicates that there is a possibility that the vehicle, in this case, Vaseline, has a protective effect against UVB exposure. However, the difference in the mean between VT and TT was significant ($p < 0.05$). Hence, it can be concluded that the protective effect of topical turmeric extract is still significantly better than the protective effect of the vehicle (Vaseline) alone. No reference explains the protective effect of Vaseline in photoaging, so further research on this needs to be done.

If the lowest type 1 collagen expression was found in SC, the highest type 1 collagen expression was found in the UVB exposure group given topical turmeric extract (TT). The results of the different tests showed that there was a significant difference in the average expression of type 1 collagen between TT and SC ($p < 0.05$). As previously explained in the discussion of TNF-α expression, curcumin in turmeric has photoprotective properties and acts as a potent antioxidant. ROS neutralized by curcumin will not activate the MAPK pathway, so type 1 collagen will not be degraded (Barzegar and Moosavi-Movahedi, 2011). In addition, curcumin is known to reduce MMP-1 expression by blocking the phosphorylation of JNK and p38 in the MAPK signaling pathway (Dunaway *et al.*, 2018; Hwang *et al.*, 2013).

One point to note is that the expression of type 1 collagen in the group given topical turmeric (TT) had a higher mean than the normal control group (NC), although it was not significantly different ($p > 0.05$). It indicates that topical turmeric extract can not only protect the dermis layer from the degradation of type 1 collagen but also increase the amount of type 1 collagen in the dermis. This result is in line with previous studies that reported that turmeric could induce collagen deposition and inhibit collagen accumulation disorders (Li *et al.*, 2016; Phillips *et al.*, 2013). More specific studies using various doses of topical turmeric extract need to be done in the future to see which topical doses are most effective in protecting the skin against UVB radiation exposure.

CONCLUSION

From the results, it could be concluded that there was a significant decrease in TNF-α expression in the skin tissue of BALB/c mice that were irradiated with UVB and given the topical *C. longa* extract compared to those not given the topical *C. longa* extract. There was also a significant increase in the expression of type 1 collagen in the skin tissue of BALB/c mice that were irradiated with UVB and given the topical *C. longa* extract compared to those not given the topical *C. longa* extract.

AUTHORS' CONTRIBUTIONS

Aletheia Threskeia contributed to the concept and design, data acquisition, drafting of the manuscript, statistical analysis, and funding. Willy Sandhika contributed to the concept and design, data analysis, critical revision of the manuscript, funding, supervision, and final approval. Retno Pudji Rahayu contributed to the concept and design, technical support, and supervision.

FUNDING

CONFLICTS OF INTEREST

The authors of this study declare that there are no conflicts of interest.

ETHICAL APPROVALS

This research was pure experimental research conducted on mice in the Faculty of Pharmacy, Universitas Airlangga, Indonesia (Ethical Clearance Certificate Number: 447/HRECC. FODM/X/2020).

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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