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PEPPERMINT LEAF EXTRACT CREAM INCREASED TRANSFORMING GROWTH FACTOR B (TGF-B) EXPRESSION AND COLLAGEN AMOUNT IN THE MALE WISTAR RAT'S SKIN EXPOSED TO UVB

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ABSTRAK

Peppermint leaves contain flavonoids, menthofuran, and other phenolic compounds that have antioxidant activity and are believed to inhibit collagen degradation in the skin. This study investigated the efficacy of a 5% peppermint leaf extract cream in increasing the transforming growth factor β (TGF-β) expression and collagen amount as indicators of collagen maintenance in male Wistar rats' skin exposed to UVB. Method A randomized post-test-only control group design was performed on male Wistar rats, local strains, aged 2-3 months, with body weights ranging from 180 to 200 grams. According to the sample calculation, 21 rats were randomly divided into three groups. The first group is the control group (without any treatment). The second group was treated with UVB without cream (UVB-only group), and the third group was treated with UVB + 5% peppermint leaf extract cream on exposed skin. After four weeks of treatment, the tissue samples were examined. The expression of TGF-β was evaluated by immunohistochemical examination, and the amount of collagen was examined by Picro-Sirius red staining. Comparative tests were performed on the measurement results of the three groups. The results showed a significant difference in the mean TGF-β expression and collagen amount among the three groups ($p<0.001$). The group exposed to UVB + 5% peppermint leaf extract cream had the highest mean TGF-β expression and collagen amount (77.41±5.79% and 82.57±2.18%, respectively), followed by the control group (63.17±12.22% and 74.05±7.15%) and the UVB-only group (50.54±11.10% and 65.65±5.56%). Administration of 5% peppermint leaf extract cream effectively increased TGF-β expression and collagen amount in the skin of male Wistar rats exposed to UVB compared to the control and the UVB-only groups.

Kata kunci: antioxidant; anti-aging; collagen; peppermint leaf; tgf-β

INTRODUCTION

In addition to intrinsic factors, aging can also be caused by extrinsic factors such as ionizing radiation, severe physical and psychological stress, alcohol consumption, poor nutrition, high sugar and carbohydrates diet, pollution, and UVB radiation (Kammeyer & Luiten, 2015; Pandel et al., 2013; Widiyanto, 2018). Excessive UVB irradiation leads to an increase in reactive oxygen species (ROS) and activator protein (AP) 1, which transcribes the enzyme matrix metalloproteinase-1 (MMP-1) in the collagen degradation process. Previous studies have shown that TGF-β plays a central role in controlling MMP production and is essential for connective tissue regeneration during wound healing. Impaired expression of TGF-β in skin fibroblasts significantly reduces type 1 procollagen gene expression, while skin resilience depends on the precise and uniform arrangement of collagen fibrils (type I and III) and elastin in the dermis (Pittayapruek et al., 2016; Shin et al., 2019). Based on these exposures, collagen level and TGF-β expression are significant predictors in skin aging assessment. The administration of antioxidants can reduce the effects of UV exposure on skin aging.

Antioxidants protect the body from damage caused by oxidation reactions induced by ROS and free radicals so that the synthesis of TGF-β is not disturbed and inhibits collagen degradation;

thus, the premature aging process does not occur (Kammeyer & Luiten, 2015; Petruk et al., 2018). Some plant extracts contain compounds that prevent ROS formation through resistance to free radicals. For this reason, many studies use natural ingredients from plants to reduce the effects of free radicals caused by UVB (Petruk et al., 2018). Previous studies have examined various plants such as ginseng, spirulina platensis, purple sweet potato, and rosella flowers that have antiaging properties by inhibiting the increase in MMP-1 expression and preventing the decrease in Wistar rat's collagen levels when exposed to UVB (Nugroho, 2020; Pratama et al., 2020).

Peppermint leaves have been widely used in herbal medicine as antiseptic, antipruritic, and carminative agents due to their high antioxidant content. Previous research has shown that Mentha piperita L. is a potent scavenger of free radicals. Peppermint leaf extract contains flavonoids, menthofuran, monoterpenes, tannins, triterpenes, sesquiterpenes, carotenoids, and minerals. This content indicates that peppermint leaves are rich in antioxidants and have the potential to prevent aging as an ingredient (Ghodrati et al., 2019). In an in vivo study, Mentha piperita L. extract was reported to have a preventive effect on mouse mesenchymal stem cells (MSCs) and showed a significant decrease in the expression of p53 and NF-kB in the cells of the group with Mentha piperita L. extract compared to the control group 11. This study aimed to investigate the efficacy of peppermint leaf extract (Mentha piperita L.) on inhibiting male Wistar rats' skin aging by increasing the expression of TGF-β and interrupting degraded collagen pathways due to UVB exposure (Sarikhani et al., 2021).

METHOD

A population of male Wistar rats (Rattus norvegicus) aged 2-3 months with a bodyweight of 180-200 grams was used for this study. The experimental study and design for this study were based on the rules and guidelines for animal research. This study is an experimental study with a randomized post-test-only control group design. Approval for all animal protocols was reviewed and approved by the Ethics Committee of Udayana University, Bali, Indonesia (Number: B/8/UN14.2.9/PT.01.04/2022). The preparation of peppermint leaf extract and phytochemical study were prepared at the Laboratory of Biochemistry and Nutrition, Faculty of Agricultural Technology, Udayana University. The cream of peppermint leaf extract was prepared in the Department of Pharmacology and Therapeutics, Faculty of Medicine, Udayana University. The peppermint leaf extract cream was peppermint leaves extracted at 5% with 96% ethanol from the preparation and added to a base cream containing methyl paraben 0.18% as much as 0.24 grams, glycerin 5% as much as 5 grams, and tween 80 1 gram.

A total of 21 male Rattus novergicus from the sample calculation were fitted for at least one week and randomly divided into three treatment groups. The first group was the group without any treatment (the control group); the second group was exposed to UVB only without any cream (the UVB-only group); the third group was exposed to UVB and received a cream containing 5% peppermint leaf extract (treatment group/study group). The cream was administered by applying an amount of 2.4 mg twice daily to the shaved back skin of Wistar rats 20 minutes before UVB exposure, four hours after exposure, and once a day when there was no exposure. UVB exposure was performed with a KN-4003 Kernel UVB lamp administered three times a week (irradiation distance 3 cm) in increments of increasing dose each week at 840 mJ/cm2 for each rat for four weeks. After treatment, skin samples with a punch biopsy with a diameter of 4 mm and a depth of 0.2 mm (full thickness) were obtained from the back skin of the Wistar rat for histological examination. TGF-β expression was examined by immunohistochemical examination and measured by counting the TGF-β-expressing fibroblast cells divided by the total number of fibroblast cells in the field of view

and multiplied by 100%. The amount of collagen was assessed by Picro-Sirius red staining and measured by the percentage pixel area of collagen- stained red compared with the pixel area of total tissue (Rozza et al., 2021). Data analysis was performed using the one-way ANOVA to compare results between groups. Post-hoc LSD was performed to assess significance in each group. Significant differences between groups were found at p <0.05 using the 95% confidence interval (CI).

RESULT

Descriptive results of TGF-β expression and collagen amount showed that the treatment/study group (which received a cream containing 5% peppermint leaf extract) had thehighest mean TGF-β expression and collagen amount, followed by the control and the UVB- only groups, respectively (Figure 1.).

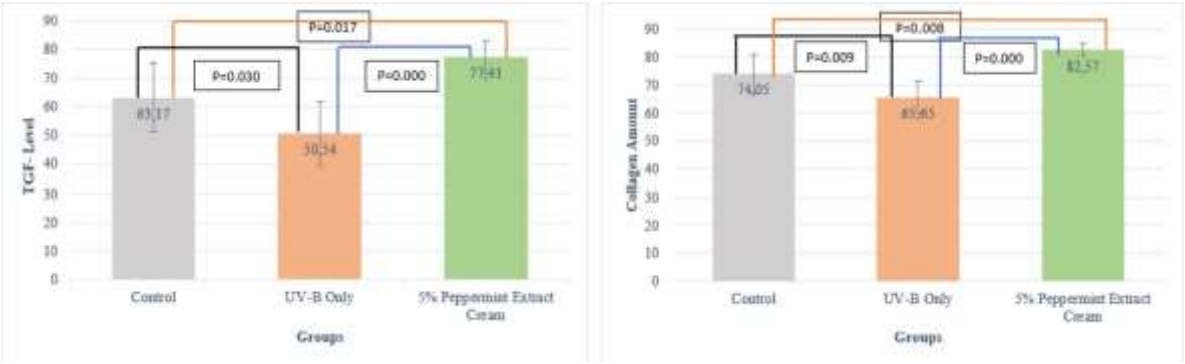


Figure 1. The Comparison of TGF-β Expression (%) and Collagen Amount (%) BetweenGroups

The ANOVA analysis results showed a significant difference between the expression ofTGF-β and collagen amount among the three groups (p<0.001). The LSD test results showed that the mean TGF-β expression in the treatment/study group was significantly higher than thecontrol group (77.41 vs. 50.54) and the UVB-only group (77.41 vs. 63.17) with p=0.017 (95%CI 2.895-25.591) and p<0.001 (95% CI: 15.524-38.219), respectively. Similar results were found for the amount of collagen. The results of the LSD test showed that the mean collagen amount was higher in the treatment/study group than in the control and UVB-only groups (Figure 1.). The results showed that 5% peppermint leaf extract cream (*Mentha piperita L.*) inhibited the reduction of TGF-β expression and collagen amount in the skin of male Wistar rats (*Rattus norvegicus*) exposed to UVB.

Histology

Histologic examination was performed at 400× magnification. In the results of the TGF- β examination (Figure 2.), it was found that the fibroblast cells expressing the most TGF-β were in the treatment/study group compared with the control and UVB-only groups. At the same time, observation of collagen amount showed damage to the collagen structure with red collagen fibers that looked thin in the UVB-only group. In contrast, collagen fibers were the thickest and most expansive in the treatment/study group (Figure 3.). This condition indicates an improvement of collagen fibers in the treatment/study group after UVB exposure.

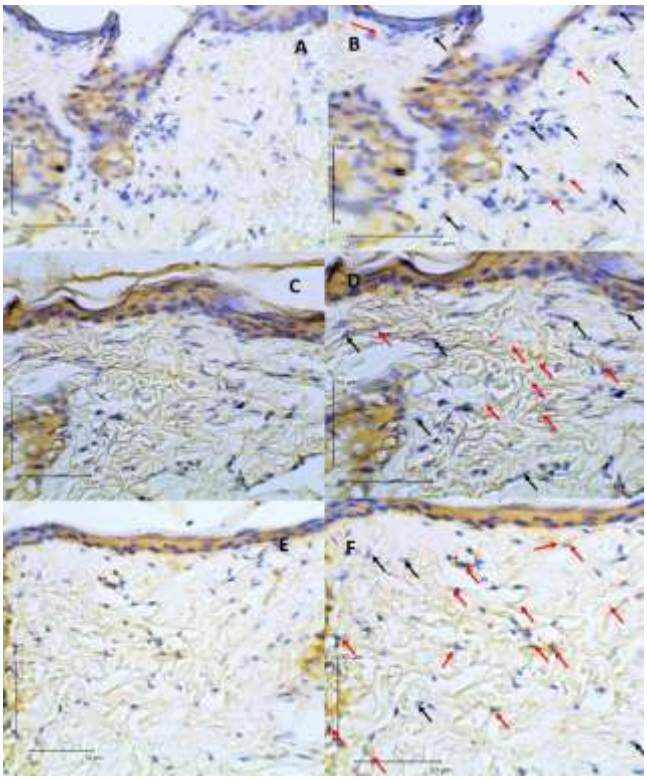
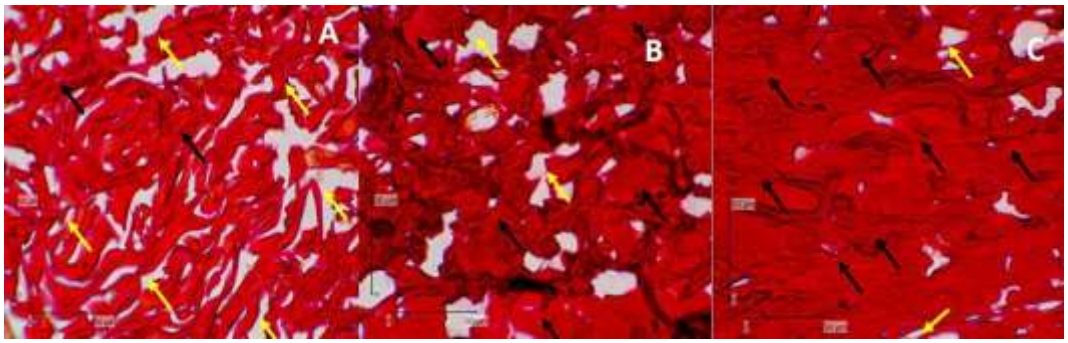


Figure 2. Expression of TGF-β in dermis tissue by immunohistochemical staining

A) UVB-only group. TGF-β expression (brown color) appears to be less than in D and more than in F. B) Magnified image of UVB group (400x). C) Control group. TGF-β expression (brown color) was higher in the control group than in the UVB-only group but still lower than in the treatment/study group. D) Image of control group magnified (400x). E) Treatment/study group. The expression of TGF-β (brown color) is most increased compared to the other groups. Red arrows indicate fibroblast cells expressing TGF-β. Black arrows indicate fibroblast cells that do not express TGF-β. F) The image of the treatment/study group is magnified (400x).

Figure 3. The collagen amount in dermis tissue with Picro-Sirius Red staining.



A) UVB group only. Damage to collagen composition and structure with red collagen fibers looking thin. B) Control group. The amount of collagen with red collagen fibers looks broad and thick but less than the treatment/study group. C) Treatment/study group. The amount of collagen with red collagen fibers looks wider and thicker. Black arrows indicate intact collagen fibers. Yellow arrows indicate incomplete collagen fibers.

DISCUSSION

UV radiation can damage human skin tissue and cause premature skin aging (photoaging). Destruction of the structural integrity of the extracellular collagen matrix, especially collagen type 1, is the main reason for the appearance of wrinkles in photoaging skin. UV-B radiation decreases type 1 collagen via two molecular pathways: stimulating collagen degradation and inhibiting type 1 procollagen production. The primary regulator that plays a role in collagen synthesis in the skin is TGF-β 1,4 . TGF-β acts by binding to specific receptor complexes, including the TβRI and TβRII receptors on the cell surface12. UVB radiation has been shown to impair TGF-β/Smad signaling by downregulating TβRII transcription, resulting in decreased procollagen synthesis in human skin fibroblasts. In addition, because of UV exposure, ROS can increase the activation of NF-kB and AP-1 and inhibit the synthesis of TGF-B (Ansary et al., 2021; Gromkowska-Kępka et al., 2021).

The results of this study are consistent with previous studies that UV radiation can reduce the amount of TGF-β. The experimental animals in the control group had higher TGF-β levels than those exposed to UV-B irradiation. These results suggest that UV-B irradiation mimics the effects of light aging in humans. Significant differences were also found in the experimental group receiving peppermint leaf extract cream (Mentha piperita L.) between the UV-irradiated and control groups. These results suggest that peppermint leaf extract cream (Mentha piperita L.) may have an antioxidant effect on the skin, as indicated by preserved and increased TGF-β levels compared with the skin tissue of the control groups. Extracts of Mentha piperita L. leaves have shown intense antioxidant activity. The main constituent of peppermint leaves is phenolic compounds, carvacrol, and thymol compounds with antioxidant properties, which have the potential for oxidative damage therapy.

The previous phytochemical analysis revealed high amounts of thymol in Mentha piperita L. (18.3 mg/g extract) (Sarikhani et al., 2021). Studies conducted in Iran show that peppermint (Mentha piperita L.) has potent antioxidant activity in Iran. Mentha piperita L. extracts from different alcoholic solvents were found to have different levels of antioxidant activity. This is due to many flavonoids and phenolic compounds in peppermint leaves (Farnad et al., 2014) . The high content of flavonoid and phenolic compounds from Mentha piperita L. may increase TGF-β levels by directly inhibiting the effects of free radicals that reduce TGF-β production. In a study conducted by Amirzade-Iranaq et al (amirazade et al., 2022). on the effect of topical application of Mentha piperita L. extract on TGF-β expression, a significant increase in TGF- β levels was observed. The study by Modarresi et al (Modarresi et al., 2019). investigating the effect of topical application of Mentha piperita L. leaf essential oil extract showed similar results, where TGF-β levels also increased TGF-β1 levels play a role in fibroblast activation and cell proliferation.

Excessive UVB irradiation increases ROS and AP-1, thus increasing MMP enzymes' transcription in the collagen degradation process. AP-1 inhibits collagen synthesis by inhibiting the type 2 receptor of TGF-β. Elevated levels of AP-1 increase the degradation of collagen (Ansary et al., 2021; Kammeyer & Luiten, 2015; Pittayapruek et al., 2016). There were differences in collagen expression in this study between the group receiving peppermint leaf extract cream (Mentha piperita L.) and the control group. The peppermint leaf extract cream (Mentha piperita L.) was able to inhibit the decrease in the amount of collagen more than the control group. Studies with similar results showed that the microscopic appearance of wounds in the study group that received peppermint extract had more collagen fibers than the inner control group. The experimental group showed differences in re-epithelialization ($P<0.001$), collagen deposition ($P<0.001$), and granulation tissue maturation ($P=0.02$) compared to the

control group. In addition, topical application of Mentha piperita L. extracts shortened the inflammatory phase, reduced inflammatory cells such as neutrophils, and increased macrophages (Hasnain et al., 2020). These changes caused an increase in angiogenesis and accelerated wound healing in the experimental group compared with the control group ($P < 0.05$) (Unalan et al., 2019). In another parallel study, a topical application was evaluated on wound healing in an infected mouse model. It was found that the rate of fibroblast migration, collagen production and secretion, and epithelial tissue regeneration increased in the Mentha piperita L.-treated group compared to the control group (Modarresi et al., 2019).

The main component of peppermint leaves is an essential oil consisting of menthol and other monoterpenes, including menthone (10-40%), menthyl acetate (1-10%), menthofuran (1- 10%), cineol (eucalyptol, 2-13%), and limonene (0.2-6%). The predominant menthol compound (33.59%) can trigger epithelial regeneration by inducing fibroblast migration at the wound site, triggering collagen formation (Unalan et al., 2019). Menthol glycoside, a menthol derivative, plays a vital role in collagen synthesis. Menthol glycoside produces new collagen, which migrates into the wound (Vilela et al., 2021). The menthone content of 21.12% in mint leaves is similar to menthol. The mechanism of action of menthone is to increase the number of fibroblasts by increasing fibrocyte activation. Fibrocyte activation results in more fibroblasts migrating into the wound, accelerating healing (Unalan et al., 2019).

One study showed that the granulation tissue formed in the menthol-treated group almost returned the epithelium to normal by day 7. The effect was more pronounced at 14 days than at seven days when epidermis formation was on the wound surface, and the margin grew toward the center. Fibroblast cells and collagen fiber deposition were found in the dermis, indicating the effectiveness of extracellular matrix remodeling (Rozza et al., 2021). The proliferative phase begins when fibroblasts activate inflammation and produce collagen and glycosaminoglycans. In other words, fibroblasts are the builders of new tissue. In extensive skin injuries, a drastic reduction in fibroblasts is the cause of delayed or failed skin repair. In addition, the secretion of chemotactic substances by platelets and macrophages stimulates the growth of new blood vessels from the wound; therefore, chemotactic agents can stimulate angiogenesis. Peppermint leaf extract aids this process by inducing angiogenesis and epithelialization. In the experimental group, wound thickness decreased in a shorter time by day 14 due to peppermint leaves. In addition, healing power and wound closure developed faster in the group receiving peppermint leaves than in the control group (Chen et al., 2015) .

Study Limitations

This study has several limitations, including that this study was based only on the tissue results due to the limited cost of the study, although histologic parameters can also describe treatment outcomes well. Another limitation is that collagen, and TGF-β levels can be examined only after treatment (posttest-only), so it is impossible to assess how quickly the peppermint leaf extract affects the given treatment. However, this design was chosen to minimize the subject's injury.

CONCLUSION

Administration of peppermint leaf extract cream (*Mentha piperita* L.) significantly increasedthe transforming growth factor-B (TGF-β) expression and the amount of collagen in the skinof male Wistar rats (*Rattus norvegicus*) exposed to UVB.

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