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The Administration of 3% Tamarind (*Tamarindus indica*) Leaves Extract Cream Inhibited the Increase of MMP-1 Expression and the Decrease of Dermal Collagen in Male Wistar Rats (*Rattus norvegicus*) Exposed to UV-B

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Abstract: ***Background:*** ***Skin aging is a complex process and influenced by intrinsic and extrinsic factors. The combination of these two factors have an impact on the dermis and wrinkle is the most common clinical symptoms. The main extrinsic factor that accelerates aging is ultraviolet exposure from sunlight and known as photoaging. Natural antioxidants are one option to inhibit aging process and tamarind (Tamarindus indica) leaves have potential because of its antioxidant content. This study was conducted to investigate the potential of 3% tamarind leaves extract cream (Tamarindus indica) in inhibiting the increased of MMP-1 expression and the decrease of dermal collagen in male Wistar rats (Rattus norvegicus) exposed to ultraviolet B.*** ***Method:*** ***This study was conducted with a post-test only control group design. Ultraviolet B irradiation given three times a week with a total dose of 840 mJ/cm^{2}for four weeks. The control group received cream base without extract as placebo, while the treatment group received 3% tamarind leaves extract cream. The cream was given twice daily, before and after UV-B irradiation. The expression of MMP-1 was examined using immunohistochemical techniques and the amount of dermal collagen was observed with Picro Sirius Red staining. The results obtained were processed using the software.*** ***Result:*** ***The significance analysis carried out by an independent sample t test showed that the mean result of MMP-1 expression in the control group was significantly higher than the treatment group (10.22 ±2.05 vs 3.71 ±0.94, p 0.001). In contrast, the mean amount of dermal collagen in the control group was significantly lower than the treatment group (61.99 ±4.98% vs 81.16 ±4.17%, p 0.001).*** ***Conclusion:*** ***It can be concluded that the administration of 3% tamarind leaves extract cream (Tamarindus indica) inhibited the increase of MMP-1 expression and the decrease of dermal collagen in male Wistar rats (Rattus norvegicus) exposed to ultraviolet B rays.***

Keywords: tamarind leaves, *Tamarindus indica*, MMP-1, collagen, ultraviolet B

1. Introduction

Skin plays an important role in a person's appearance and is often used as a parameter that can reflect age so people are competing to rejuvenate the skin to make it look more attractive, in the other hand, we all through the ageing process.

Aging is a complex progressive process that can be prevented, slowed down, and returned to its youthful state. If the organ function can be maintained, people will look younger than their peers and have a better quality of life.[1]

Skin aging itself is also acomplex process and influenced by intrinsic and extrinsic factors. The combination of these two factors will have an impact on the dermis and one of the most common clinical symptoms is wrinkles.[2] Ultraviolet (UV) is the main extrinsic factor that accelerates this process and known as photoaging.[3]

Ultraviolet exposure will cause major changes on the skin such as triggering a signal transduction pathway due to excess reactive oxygen species (ROS). This accumulation will induce matrix metalloproteinase (MMP) expression.[4,5] Matrix metallo-proteinase such as collagenase causesextracellular matrix (ECM) degradation, especially collagen,and lead to wrinkle formation.[5]

Currently, photoaging prevention strategies focus on increasing the body's antioxidant defenses. Antioxidants are substances that can prevent or slow down the oxidation of other molecules. The protective function of antioxidants occurs through suppression of free radical production, the role as a scavenger, and triggerbiosynthesis of other antioxidant components so that the harmful effects can be inhibited and minimized.[6] Natural antioxidant from herbal is one of the options and considered relatively safer.[5]

*Tamarindus indica*or tamarind leaves contain flavonoids, polyphenols, tannins, and ascorbic acid.[7,8,9] Those ingredients have many benefits and play a synergistic role in increasing the activity of antioxidant enzymes and suppress the harmful effect of ROS.[8]

If the skin aging process can be prevented by the antioxidant, the quality of life will improve, and based on that,we are interested to study 3% tamarind leaves extract cream effect on the skin through MMP-1 expression and dermis collagen after UV-B exposure.

2. Method

Study design and sample

This study was conducted with a post-test only control group design and used 36 male Wistar rats as research subjects. Subjects were healthy rats aged 10 - 12 weeks old and weigh 180 - 200 grams. Subjects were randomly divided into two groups, control and treatment groups (n=18).

Subject intervention

Ultraviolet B irradiation with a total dose of 840 mJ/cm^2 was applied three times a week for four weeks.The hair was shaved around the intervention area to optimized penetration and cream was given twice daily, before and after irradiation.The control group received a cream base without extract as a placebo and the treatment group received 3% tamarind leaves extract cream. The creams were also applied on days without irradiation.After four weeks, all rats were anesthetized, and then a skin sample was obtained from the back of each rat by punch biopsy.

Measurement of MMP-1 expression

The expression of MMP-1 was examined using immuno-histochemical techniques. The stained samples were divided into three fields of view and observed using a light microscope with 400x magnification. Matrix metallo-proteinase-1 expressions calculated as the mean of the MMP-1 expressing fibroblast cells (brown-stained cytoplasm). The results then analyzed using an independent t-test.

Measurement of dermal collagen

The samples were stained with Picro Sirius Red and observed using a light microscope with 40x magnification. The sample's pictures were analyzed with Adobe Photoshop CS3 and Image J. The percentage of dermal collagen was obtained by dividing the collagen pixel area with the total pixel in one field of view. The results then analyzed using an independent sample t-test.

3. Result

The microphotographs are shown in **Figure 1.** It clearly shows the difference between control and treatment groups. The MMP-1 expression was lower in the treatment group and the density of dermal collagen was higher in the treatment group.

The data between treatment and control group were analyzed using an independent sample t-test and the mean differences between groups are shown in **Table 1.** The mean expression of MMP-1 was significantly higher in the control group than the treatment group, 10.22 ±2.05 vs 3.71 ±0.94 respectively (p = 0.001).In contrast, the mean amount of dermal collagen in the control group was lower than the treatment group (61.99 ± 4.98% vs 81.16 ± 4.17%, p = 0.001).

Table 1. Comparison of MMP-1 expression and collagen dermis between control and treatment group

Variables	Group	n	Mean ± SD	Mean Difference (CI 95%)	p
MMP-1 expression	Control	18	10.22 ± 2.05	6.51 (5.41 – 7.61)	0.001
	Treatment	18	3.71 ± 0.94		
Collagen dermis (%)	Control	18	61.99 ± 4.98	-19.17 (-22,28 – -16.06)	0.001
	Treatment	18	81.16 ± 4.17		

*SD : standard deviation, CI : confident interval

4. Discussion

This study confirmed the hypothesis that 3% tamarind leaves extract cream can prevent skin photoaging, proven by the lower MMP-1 expression and higher dermal collagen in the treatment group.

More than 90% of skin changes are caused by extrinsic factors, one of them is sunlight. Ultraviolet exposure will stimulate ROS generation and cumulative exposure for ten weeks will cause significant wrinkle. There will be also fibroblast damage marked by collagen degradation and decrease collagen synthesis.[6] Harmful effect of UV irradiation also marked by increases in MMP-1 regulation and as we know that this protease plays important role in collagen degradation.[10]

Plants are naturally producing useful secondary metabolites and tamarind leaves has long been used as wound healing, acne, fever, and abdominal traditional treatment.[11,12] Our phytochemical result shows that tamarind leaves had phenol, flavonoid, tannin, and vitamin C constituents that are potentially useful as an antioxidant to inhibit skin damage caused by UV.

We found that MMP-1 expression in the control group was significantly higher than the treatment group (10.22 vs. 3.71, respectively). This shows tamarind leaves role in reducing the adverse effect of UV exposure as an antioxidant in which phenol, flavonoid, and tannin plays in electron transfer mechanism and as a metal chelating agent to reduce ROS accumulation.[13,14]

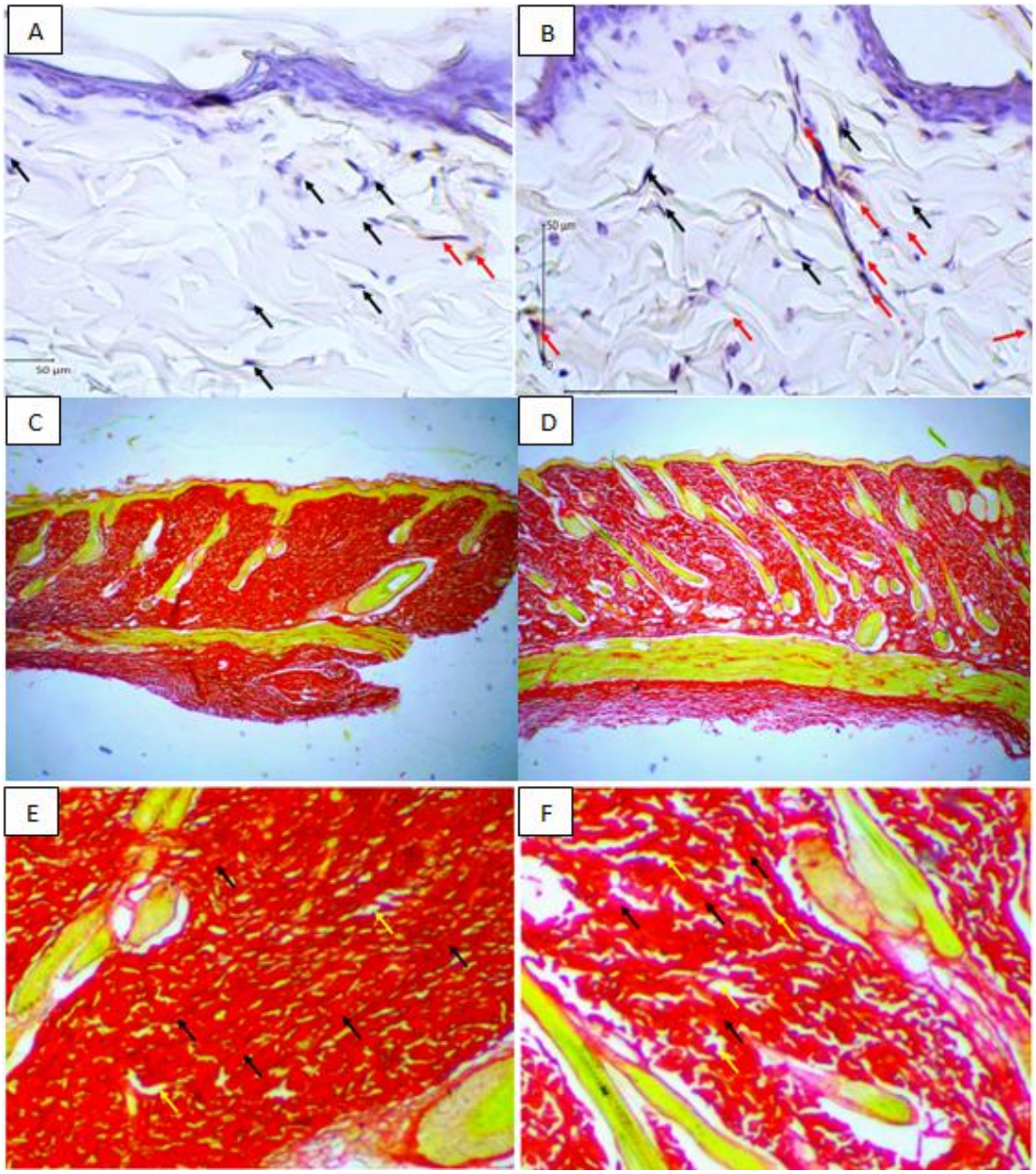

Figure 1. Figure **A** (treatment group) and **B** (control group) show immunohistochemistry staining of MMP-1 expression in rat dermal tissue with 400x magnifications. Black arrow indicates non-expressing MMP-1 fibroblast and red arrow indicates fibroblast that express MMP-1. Treatment group had less MMP-1 expression. Figure **C** (treatment group) and **D** (control group) show Sirius red staining of collagen expression in rat dermal tissue with 40x magnifications. Figure **E** (treatment group) shows more intact and dense collagen fibers than Figure **F (**control group). Black arrow indicates intact collagen fibers and yellow arrow indicates damage collagen fibers.

Collagen synthesis is reduced significantly in 12 hours after UV-B exposure and to prevent this process we can utilize vitamin C.[10] The benefit of vitamin C in tamarind leaves is already proven in our study. The treatment group had significantly higher dermal collagen compared to the control (81.16% vs. 61.99%, respectively).

This vitamin is an important component in collagen biosynthesis as a prolyl and lysyl hydroxylase cofactor in which both of them are important enzymes in collagen fiber stabilization. Daily application for four months may increase collagen gene expression, tissue inhibitor MMP-1 synthesis, and papilla dermis density.[15] The other antioxidant components in tamarind leaves are support vitamin C benefits to maintain dermal collagen.

5. Conclusion

Our study proves the anti-aging potential from 3% tamarind leaves extract cream through its MMP-1 inhibitor activity and prevent dermal collagen reduction caused by UV-B irradiation in male Wistar rats.

Ethical Approval

This study had been ethically approved by the ethical commission of the Faculty of Medicine Udayana University with approval letter number 38/UN14.2.9/PT.01.04/2020.

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