RESEARCH ARTICLE

Indonesian Journal of Anti-Aging Medicine
Volume 4, Number 1, January - June 2020: 1-4

IJAAM
Indonesian Journal of Anti Aging Medicine

DOI : 10.36675/ijaam.v4i1.39

Topical administration of *deep sea shark liver oil* (*desslo*^{tm}) inhibited mmp-1 expression in rat's skin exposed to ultraviolet-B

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Introduction: Squalene is one of strong antioxidants that can inhibit aging proses. However, its effect on the skin aging is still understudied. Therefore, the purpose of this study was to assess the efficacy of topical administration of Deep Sea Shark Liver Oil toward MMP-1 expression in Wistar rat's (*Rattus Norvegicus*) skin exposed to UVB.

Methods: An experimental randomized post-test only control group study was conducted using a total of 36 rats Wistar rats were used which were divided into 2 groups. The control group received placebo solution while the treatment group was treated by Deep Sea Shark Liver Oil solution. All groups were exposed to UVB with a total dose of 840 mJ/cm^2 for 4 weeks. Biopsy was conducted for skin sample collection for the examination expression of MMP-1.

Results: Immunohistochemistry evaluation showed that the dermal MMP-1 expression in the control group was higher than in control group. According to the percentage of expression analysis, the mean of MMP-1 expression in control group were significantly higher (29.53%) compared to the treatment group (9.72%) (p=0.001).

Conclusion: It can be concluded that the topical administration of Deep Sea Shark Liver Oil solution inhibited the expression of MMP-1 in rat's skin exposed to UVB.

Keywords: Deep Sea Shark Liver Oil, MMP-1, UVB

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Submitted: 5 January 2020
Accepted: 11 May 2020
Published: 1 June 2020

INTRODUCTION

The main external factor that contributes to photoaging is ultraviolet-B (UVB) exposure.[1] UVB induced free radicals formation which then caused DNA damage by forming thymidine cross-link and reactive oxygen species (ROS). Consequently, ROS induced cellular and molecular damages as well as activating cellular signalling (Tumour Necrotizing Factor Alfa [TNF-α] associated signalling) that increased MMP-1,-3, and -9 expression. These proteinases increase collagen degradation which results in decreased tensile strength and wrinkles.[2,3] Among those 3 MMPs, MMP-1 specialized in collagen and elastin degradation. There is a report which stated that even short term UV radiation was enough to induce a marked increase in MMP-1 expression.[4] Therefore, inhibiting MMP-1 is a sensible approach in inhibiting photoaging of the skin.

Deep Sea Shark Liver Oil is known to contain sufficient antioxidants which can prevent oxygen singlet and protect skin surface from lipid peroxidation caused by UV radiation. Deep Sea Shark Liver Oil contains approximately 89% Squalene, which is has a natural cholesterol precursor triterpeneoid structure. In nature, squalene can be found in several daily food items such as pumpkin, olive oil, nut oil, and grain oil, but dogfish-shark liver oil has been known to have the highest concentration.[5] However, there is still few studies that evaluate its topical effect on skin aging.

Squalene has attracted lots of attentions since its discovery in shark liver extract by Dr. Tsujimoto in 1903. Squalene has polyunsaturated hydrocarbon chain with 6 isoprene unit called Triterpene. Another Japan scientist demonstrated that squalene could prevent lipid peroxidation caused by UV radiation. Therefore, this study aimed to evaluate the effect of topical administration of Deep Sea Shark Liver Oil solution toward dermal MMP-1 after UVB ray exposure.

METHODS

Study design and sample

An experimental randomize post-test only control grup study was conducted using 36 wistar rats (*Rattus Norvegicus*) aged 48-week-old and weighed 160-180 gr which were randomly divided into 2 groups (n= 18). The rats were acclimated to the laboratory environment for 7 days before the start

of the experiment.

Subject Intervention

All rats were exposed to UV-B rays at a frequency of 3 times/week for 4 weeks with a total dose of 840 mJ/cm^2 per rat. In addition to UV-B exposure, the treatment group received topical Desslo™ while the control group was only treated by topical glycerine cream. After 4 weeks, all rats were anesthetized and then skin sample was obtained from each rat by excising 1cm x 1cm x 4mm of back skin tissue for MMP-1 assessment through immunohistochemical staining.

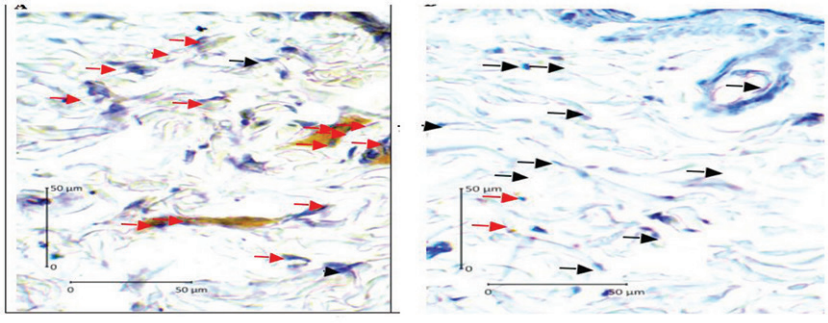
Measurement of MMP-1 expression

MMP-1 expressions were assessed by using was performed using digital analysis method. The stained sample was observed by using light microscope at 400 times magnification. A photograph was obtained (JPEG format) and assessed using Optilab Viewer 1.0 software as well as Image Raster 2.1 (Miconos, Indonesia). MMP-1 was expression was calculated as the ratio of MMP-1 expressing fibroblast cell to MMP-1 non-expressing fibroblast cell. The results were then analyzed and presented with descriptive analysis and comparative testing using independent t-test.

Table-1. MMP-1 average between test group

Test Group	n	MMP-1 average expression (%)	SB	*F*	P
Po	18	29.53	4.32	3.216	0.001*
P1	18	9.72	2.21		

Note: *show a significant result (p<0.05)



Control Group **Treatment Group**

Figure 1. Immunohistochemistry staining of MMP-1 in rat skin showed increased expression of MMP-1 in control group compared to treatment group. Control group was exposed to UVB and treated with glycerin placebo while the treatment group was exposed to UVB and treated with topical Desslo™ oil. The red arrow indicated fibroblast cell nucleus expressing MMP-1 (brown stain). The black arrow indicated fibroblast cell nucleus without MMP-1 expression (stained as brown).

RESULT

Immunohistochemical staining showed that the control group had higher expression of MMP-1 compared to treatment group (Figure 1). This finding was confirmed by analyzing the picture from all samples digitally. The analysis showed that the average expression of MMP-1 in control group was 29.53±4.32 while in treatment group, it was 9.72±2.21. The difference between those group was statistically significant (p<0.05) (Table 1).

The results showed that the MMP-1 expression in the control group (P0) was 29,53±4,32 and treatment group (P1) was 9,72±2,21. T-independent test on this result would value P of 0,001. This means that there has been a significant decrease of MMP-1 expression at test group which has been given Desslo™ topically with p<0.05 (Table 1 and Graphic 1). Histopathologically can be showen at Figure 1.

DISCUSSION

This study confirmed the hypothesis that topical application of Desslo™ can prevent dermal photoaging. The possible mechanism that we showed here was the inhibition of MMP-1 expression. The dose of Desslo™ used in this study was 0.8 ml dosage which was derived from preliminary research which showed there was no increase of MMP-1 expression on rat's skin after radiated by UVB.[7] Dermis layer of skin exposed to UV-B produce high amount of ROS which caused oxidative stress and photoaging.

The formation of ROS occurred within less than 30 minutes after UV radiation and peroxide level increase doubled in human skin. NADPH oxidation was also observed in keratinocytes which indicated an ongoing molecular oxygen reduction into anion hydroxide. Hydrogen peroxide reacted further with radical hydroxyl which resulted in DNA, protein and lipid damage and activate adverse signaling pathway.[8]

In this study, we demonstrated that topical application of Deep Sea Shark Liver Oil prevented dermal MMP-1 increase after UV-B exposure. UV-B exposure is known to initiates molecular signaling which damage the skin tissue structures. Other

than that, UVB also produces ROS,[9] which activate surface receptors such as EGF (*epidermal growth factor*), IL-1 (*interleukin-1*), insulin, *keratinocyte growth factor*, and TNF-α (*tumor necrotizing factor-α*). These cellular signaling stimulates Mitogen Activated Protein (MAP) kinase p38 and c-Jun amino terminal kinase (JNK) activation which then induce MMP transcription and decrease procollagen I and III expression. Additionally, TGF-β receptor expression is also decreased which slows extracellular matrix formation.

Desslo™ contains *Squalene* which has triperpene molecule with 5 active chemical structure and has as an antioxidant effect. DPPH test at Airlangga University showed that Desslo™ has 0.24 times capacity to slow down ROS and MMP-1 formation compared to vitamin-E. However, despite its lower antioxidant capacity, in vivo analysis showed that Deep sea shark liver oil has a good effect in inhibiting MMP-1 expression in wistar rat's skin which means it inhibited inflammatory processes caused by UV-B radiation and prevented photoaging.

Additionally, Desslo™ contains omega-3 which has long and short chemical chain. It has been proved to be able to protect skin tissues from radiation, reduce inflamation from UV radiation and also inhibited cell transformation by reducing AP-1 activity which will also stimulates MMP-1, MMP-2 and MMP-9 expression. Topical omega-3 study has also been done and resulted in reduced epidermal thickness and collagen upon UV radiation.[10,11] Finally, Desslo™ also contains Beta-carotene which has antioxidant and radical scavenger effects. Protection effect of betacaroten against UV radiation has been reported and it was able to reduce wrinkles and increase skin elasticity via increased procollagen expression.[12]

All of the compositions of Desslo™ have antioxidant activity which neutralizes singlet oxygen by binding to transitional metal ions and preventing the formation of free radicals. Squalene is known to have low ionization threshold, so it easily gives electrons without experiencing molecular disturbance.[13] Therefore, Desslo™ can be considered as one of potential choices to prevent dermal oxidative damage due to UV-B exposure and other sources of oxidative damage.

CONCLUSION

It can be concluded that topical application of Deep Sea Shark Liver Oil (Desslo™) solution prevents MMP-1 expression in Rats skin after UVB exposure.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

FUNDING

This study was self-funded without any contribution from third party.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 401/KE-PH-Lit-2/VII/2019

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