

## Topical administration of deep sea shark liver oil (DESSLO™) inhibit Nuclear Factor-kappa Beta (NF-κB) expression in Wistar rats (*Rattus Norvegicus*) skin exposed to ultraviolet-B

Devita Horax<sup>1\*</sup>, A. A. G. P. Wiraguna<sup>2</sup>, Gde Ngurah Indraguna Pinatih<sup>3</sup>

**Introduction:** Repeated exposure of the skin to UV-B rays induce reactive oxygen species (ROS) formation that activates Nuclear Factor-kappa Beta (NF-κB) and stimulates pro-inflammatory cytokines expression which leads to premature aging of skin. Deep-sea shark liver oil contains ±80% squalene which could help neutralized ROS. Therefore, the purpose of this study was to assess the effect of topical deep sea shark liver oil toward NF-κB expression in UV-B-exposed on wistar rat (*Rattus norvegicus*) skin.

**Methods:** A true experimental posttest-only control study was conducted using 36 Wistar rats which divided into 2 groups ( $n=18$ ). Control group was exposed to UV-B and treated with topical placebo basis of glycerin

while the treatment group was treated with topical deep sea shark liver oil. A total of 840 mJ/cm<sup>2</sup> UV-B exposure was given for 4 weeks. NF-κB expression was examined by immunohistochemistry.

**Results:** The mean NF-κB expression was significantly higher in control group at  $30.8 \pm 3.81$  while the treatment group treated with topical deep sea shark liver oil has average expression at  $10.4 \pm 1.98$ . The independent *t*-test showed a *t*-value of 20.090, and a *p*-value of 0.033.

**Conclusion:** It can be concluded that topical deep sea shark liver oil inhibits the expression of NF-κB expression in UV-B-exposed Wistar rat (*Rattus norvegicus*) skin.

**Keywords:** Deep sea shark liver oil, NF-κB, Ultraviolet B, Skin

<sup>1</sup>Master Program in Biomedical Sciences, Anti-Aging Medicine Concentration; Faculty of Medicine, Universitas Udayana, Denpasar, Bali

<sup>2</sup>Department of Dermatology and Venerology, Faculty of Medicine, Universitas Udayana, Denpasar, Bali

<sup>3</sup>Department of Public Health, Faculty of Medicine, Universitas Udayana, Denpasar, Bali

### INTRODUCTION

Aging is a natural process experienced by all cells and primarily marked by continuous decrease in physiological function. There are several factors that influence aging, but ultraviolet B (UV-B) radiation play a great role in premature aging on the skin, which also known as photoaging.<sup>1</sup> Long and repeated UV-B exposure results in DNA damage and reactive oxygen species (ROS) formation that trigger the activation of Nuclear Factor-kappa Beta (NF-κB), inducing proinflammatory cytokines production in the skin and eventually cause premature aging of the skin.<sup>2</sup>

In order to better neutralize free radicals, external antioxidants is needed. Squalene was firstly discovered in 1906 by Mitsumaru Tsujimoto, a Japanese industrial engineer.<sup>3</sup> Deep-sea shark liver oil contain squalene which is work on the skin by absorbing singlet oxygen and protecting the skin surface from lipid peroxidation due to UV radiation. Squalene is not only synthesized at the cellular level but can also be consumed. Other than shark liver oil, squalene can also be found in many

sources such as pumpkin, olive oil, peanut oil, and wheat seed oil. But the most abundant content of squalene is found in the oil extracted from deep sea shark liver.<sup>3</sup>

Deep-sea shark liver oil has long been known and consumed, but there have been no specific studies on its effects toward photoaging of the skin. Therefore, this study was aimed to assess the effect of topical deep sea shark liver oil toward NF-κB expression which represents photoaging of the skin.

### METHODS

#### Study Design and Sample Selection

This study was an experimental research using post-test only control group design. The subjects were thirty-six Wistar rats (*Rattus Norvegicus*), 48-week-old, 160-180 gr in weight, which were randomly divided into 2 groups ( $n=18$ ).

#### Subject Intervention

The subject was adapted for 7 days and then divided into 2 groups. The first group was a control group which exposed to UV-B and treated with topical

#### \*Corresponding Author:

Devita Horax; Master Program in Biomedical Sciences, Anti-Aging Medicine Concentration; Faculty of Medicine, Universitas Udayana, Denpasar, Bali;  
devitahorax@yahoo.com

Submitted: 8 January 2020

Accepted: 15 April 2020

Published: 1 June 2020

placebo basis of glycerin. On the other hand, the treatment group was exposed to UV-B and treated with topical deep sea shark liver oil. A total of 840 mJ/cm<sup>2</sup> UV-B exposure was given for 4 weeks at frequency of 3 times a week. After 4 weeks, all rats were anesthetized and then 1cm x 1cm of the back skin was taken at 3-4 mm in depth for histological examination of NF-κB expression.

### Measurement of NF-κB expression

The assessment of NF-κB expressions was performed using digital analysis method with 400x microscopic magnification. The tissue slide was photographed and saved in JPEG format using the Optilab Viewer 1.0 software and Image Raster 2.1 (Miconos, Indonesia). NF-κB was counted as the ratio of NF-κB -expressing fibroblast cell to NF-κB-non-expressing fibroblast cell. The results were then analyzed statistically using independent *t*-test.

### RESULTS

The results showed that the NF-κB expression in the control group was 30.8±3.81 while the treatment

group had lower expression at 10.4±1.98. Statistical analysis using independent sample *t*-test showed that the difference was statistically significant (*p*=0.033) (Table 1). Therefore, topical application of deep sea shark liver oil effectively inhibits NF-κB expression in rat's skin exposed to UV-B.

Histological examination clearly showed that difference in NF-κB expression between the two groups. As depicted in Figure 1, NF-κB-expressing fibroblasts were more common in control group while in treatment group, they were scarcely distributed and hardly found.

### DISCUSSION

Keratinocytes on dermis layer is the target of the UV-B, ROS activation causes UV-induced oxidative stress which is the cause of photoaging.<sup>4</sup> NF-κB is activated by UV-B radiation which then triggers upregulation of proinflammatory cytokines such as IL-1, IL-6, IL-18, and TNF-α. TNF-α plays a major role in the dermal inflammation, inhibits collagen synthesis and induces MMP-9 production. Increased expression of TNF-α is associated with lower collagen production by increasing collagenase activity in fibroblasts. Meanwhile, IL-1 and IL-18 primarily induce inflammation but eventually will augment TNF-α-mediated effects.<sup>5</sup>

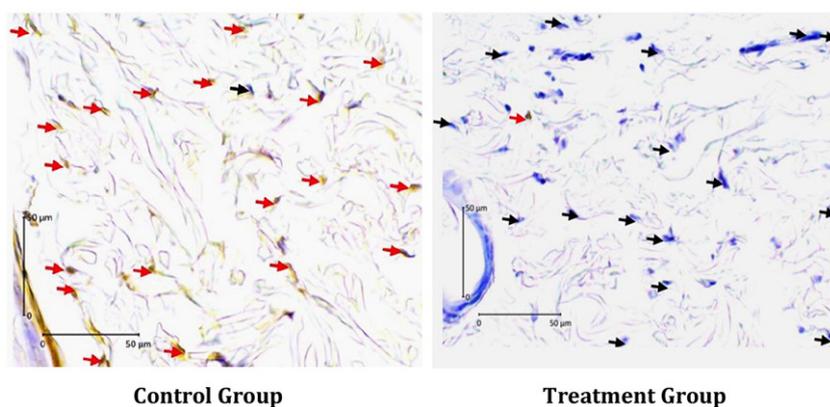
Proinflammatory cytokines upregulate MAPK signaling pathway and activate transcription factors *c-Fos* and *c-Jun* by inducing their heterodimerization. Transcription factor activator protein-1 (AP-1) is formed from heterodimerized *c-Fos* and *c-Jun* precursor. It increases the expression of matrix metalloproteinase (MMP) causing the degradation of collagen and inhibits pro-collagen synthesis. Other proinflammatory cytokines cause a decrease in skin collagen and further damages to the structural integrity of the dermis which lead to premature aging.<sup>6</sup>

Deep-sea shark liver oil has 0.24 times antioxidant activity compared to vitamin E according to on DPPH measurement at Airlangga University. Despite its lower activity, the anti-oxidant effect Deep-sea shark liver oil is still sufficient to protect keratinocytes against oxidation insults. A study from Chiayi National University, Graduate Institute of Food Science and Graduate Institute of Biotechnology in Taiwan in 2002 concerning "Squalene Content and Antioxidant Activity of *Terminalia cattapa* Leaves and Seeds", state that squalene has potent antioxidant properties that inhibit conjugated diene hydroperoxide (CDHP) formation, yet has very low DPPH antioxidant

**Table 1. The comparison of NF-κB expression between control and treatment group**

Group	n	Mean (%)	SD	t	P
Control	18	30.8	3.81	20.090	0.033*
Treatment	18	10.4	1.98		

Note: \*show a significant result (*p*<0,05)



**Figure 1.** Histopathological examination of rats skin with IHC staining. Control Group was exposed to UVB and treated with glycerin placebo; Treatment Group was exposed to UVB and treated with topical deep sea shark liver oil. The red arrow indicated fibroblast cell nucleus expressing NF-κB (stained as brown). The black arrow indicated fibroblast cell nucleus that does not express NF-κB (stained as brown).

activity. It indicated that the in-vitro antioxidant activity of squalene is low but cannot be used as an absolute indicator. Therefore another examination of the antioxidant activity of squalene is needed.

Despite the low in-vitro anti-oxidant activity of deep sea shark liver oil, in-vivo experiment showed that it has a good activity in inhibiting NF- $\kappa$ B expression in rat's skin exposed to UV-B. This result indicated that it may have a significant effect in photo-aging prevention. The squalene within deep sea shark liver oils reduces singlet oxygen by binding to transition metal ions and preventing the formation of free radicals. Therefore, squalene can be considered as an excellent antioxidant in the skin. Squalene protects the surface of the human skin from lipid peroxidation due to UV exposure and other sources of oxidative damage. Squalene has a low ionization threshold, which made it easily donate its electrons without experiencing molecular disturbances.<sup>7</sup>

### CONCLUSION

The result of this study indicated that topical deep sea shark liver oil significantly inhibited the expression of Nuclear Factor-kappa Beta (NF- $\kappa$ B) in UV-B-exposed rat (*Rattus norvegicus*) skin. Further study is needed to validate this finding and to determine the effective dose of topical deep sea shark liver oil.

### 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 403/KE-PH-Lit-2/VII/2019

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