The ethanolic extract of red cabbage (Brassica oleracea l. var, capitata f. rubra) in cream preparation to the dermal-thickness of male Wistar mice (Rattus norvegicus) after Ultraviolet-B exposure

Gayathiri Mohana Krishnan1*, IG Kamasan Arijana2, I Wayan Sugiritama2

ABSTRACT

Background: Photoaging effect in dermal layer of skin was commonly caused by Ultraviolet B (UV-B) rays exposure. This study aims to assess the histological features of the dermis layer in the male Wistar mice after applied with the ethanolic extract of red cabbage following UV-B exposure.

Methods: About 25 male Wistar rats were enrolled in this study by using post-test only control group design during 2 weeks. They were divided into 5 groups as follows: control group (P1), basic cream (P2), 5% ethanolic extract of red cabbage cream (P3), 10% ethanolic extract of red cabbage cream (P4), and 20% ethanolic extract of red cabbage cream (P5). The UV-B dosage used are 325 mJ/cm² among groups prior to study. The dermis-elastic fiber thickness was assessed by Image Raster software. Statistical analysis was carried out using SPSS version 20 in measuring normality test, mean, and ANOVA.

Results: There was no statistically significant difference in the thickness of dermal layer among groups (P = 0.3). The mean of dermis thickness was 666.60±143.15µm in P5 group, followed by 651.19±189.18µm in P4 group, and 547.43±188.41µm, 544.76±100.67µm, and 504.10±129.40 µm in P3, P2, and P1 group respectively.

Conclusion: There was no a significant difference among different dosage of the ethanolic extract cream of red cabbage to the thickness of dermal layer after UV-B exposure.

Keywords: dermal layer, ethanolic extract, red cabbage, thickness

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INTRODUCTION

Skin is divided into three main parts known as epidermis, dermis, and subcutaneous tissue. The function of the skin is to act as a barrier between the internal organ, the body and the environment. Epidermis is composed of keratinocytes that form the outer protective skin barrier. The dermis is divided to upper and lower level. The upper level is called the papillary region made of loose connective tissue while the lower level is the reticular layer. Dermis has strong collagen and elastic fibers. Subcutaneous tissue is the innermost layer of skin. It is mostly made of fats and hair follicles. It acts as cushion to protect everything beneath it. (Gragnani et al, 2014)

Aging is known merely as the process of growing old. But it is best defined as the decreased maximal function and reverse capacity in all organs giving higher chances of disease and death. (Yaar et al, 2002).

Skin aging is a continuous process determined by intrinsic aging, lifestyle and environment factors such as the exposure to sun, cigarette smoking, and body mass index. The most common elements of skin aging are sun exposure and smoking. The most visible signs of skin aging are wrinkles, atrophy, sagging, and irregular pigmentation. Another factor to prevent skin aging is balanced nutrition. This balanced nutrition is not only to prevent chronic disease but also to maintain healthy skin and ensure normal functioning. Dietary supplementation with vitamin E and C, carotenoids and polyunsaturated fatty acids play a role in giving protection to the skin against sun exposure. Many different studies have observed several other ingredients such as vegetables, fruits or even olive oil can protect the skin from sun exposure. (Cosgrove et al, 2007).

Sunlight is the primary source of UV rays. UV rays make up only a tiny portion of the sun's rays; they are the leading cause of the sun's damaging effects on the skin. UV rays damage the DNA of skin cells. There are 3 types of ultraviolet rays namely UV-A, UV-B, and UV-C. Both UVA and UVB rays can damage skin and cause skin cancer. UVB rays are a more potent cause of some skin
cancers. UVA is having the longest wavelengths (315–400 nm), UVB being mid-range (290–320 nm) and UVC being the shortest wavelengths (100–280 nm). UVB-light induces the proteolytic cleavage of collagen fibrils. Repeating damage through successive UV-irradiations is only repaired insufficiently as a result of glycosylation products which cannot be degraded. Both accumulation of these micro-lesions and additional loss of total skin collagen with age essentially contribute to the functional impairments of the aged collagen matrix, resulting in skin laxity and wrinkles. Apart from collagen, UVB has been reported to affect other dermal ECM components, in particular, hyaluronan (HA) and proteoglycans (PG). HA is an unbranched polymeric carbohydrate consisting of alternating disaccharide units (D-gluconuronic acid beta (1–3)-D-N-acetyl-glucosamine beta(1–4)). Through interaction with its receptors (e.g., CD44) and its binding partners such as tumor necrosis factor-stimulated gene 6, HA is thought to determine cellular phenotypes critically. Histological and biochemical studies of the HA metabolism reported contradictory results depending on the intensity, duration and amount of irradiations. It was shown that acute UVB-irradiation leads to an increase of dermal HA whereas studies investigating chronic irradiation reported a decrease of dermal HA. (Stuart et al., 2013)

Antioxidants are chemicals that can prevent or slow cell damage. It is a compound that can donate electrons and counteract free radicals. Natural antioxidants are usually found in fruits and vegetables. (Gragnani et al., 2014) Antioxidants from *Brassica oleracea* or also known as red cabbage plays an essential role in health of the skin. Red cabbage has ten times more vitamin A and twice as much iron as green cabbage. Based on the explanation above, this study aims to determine role of red cabbage ethanolic extract (Brassica oleracea L. var, capitata f. rubra) in cream preparation to red-cabbage ethanol, and Treatment group 5 (P5) 20% ethanolic extract cream of red-cabbage. All of groups received UV-B dose three times a week in a dose of 325 mJ/cm² prior study until 2 weeks. Identification of red cabbage has been carried out in Eka Karya Botanical Garden Bedugul while the sample processing and extraction of red cabbage will be conducted at the Laboratory of Biopesticides Postgraduate Udayana. The base and treatment cream in different dosage were applied twice daily during 2 weeks of radiation. Throughout the study period, all five groups were given an exact amount of food and water. The temperature and humidity of the experiment environment are strictly controlled. The thickness of dermis was assessed at Department of Histology, Faculty of Medicine, Udayana University using Image Raster software. The tissue sample was examined by using light microscope Olympus CX31 (Japan) in 400 times magnification, and hematoxylin and eosin (H&E) staining were performed.

The data obtained was statically analyzed using the ANOVA method by SPSS version 20. The results of mean, standard deviation, and significance test were shown. A P-value less than 0.05 was considered statistically significant.

**RESULTS**

The histological analysis was shown in Figure 1 regarding the thickness of dermis layer. Haematoxylin and Eosin (HE) stain were used and observed by using a light microscope with 10 x 40 magnification. The thickest of dermis layer was found in group P5 with 20% ethanolic extract cream of red-cabbage, followed by P4, P3, P2, and P1 groups (Figure 1). The results are also similar from Image Raster software measurement where the highest mean of dermis elastic-fiber thickness was found in group P5 (666.60±143.15µm), followed by P4 (651.19±189.18 µm), P3 (547.43±188.41 µm), P2 (544.76±100.67µm), and P1 (504.10±129.40µm) (Table 1).

The thickness of the dermis layer in male Wistar rats was then analyzed by using one way ANOVA method. The results found that there was no significant difference between the thicknesses of the dermis among groups (P = 0.39) (Table 1).

**DISCUSSION**

The red cabbage (Brassica oleracea L. var, capitata f. rubra) is known possess a high levels of antioxidant metabolites associated with beneficial skin health effects, including vitamins (especially vitamin A, C, E, K and B-6), carotenoids (such as γ and β-carotene and zeaxanthin), anthocyanins, folate,
and UVB burnt cells (apoptotic cell) formations have been predominantly observed after UVB exposure. Long term UVB exposure leads to a decline in the regulation of the hyaluronic acid synthase (HAS) enzyme, which causes damage to fibroblast. Besides that, a study conducted by Hassan in 2015 showed that the penetration of UVB rays on the skin only reaches the epidermal layer. However, the thickening of the dermis layer is due to the spacing of the cells between the delivery of fluids, dissolved substances, and circulating blood cells into the interstitial tissue to the injury site during inflammation. It is also due to the release of histamine by mast cells in the dermis layer. The inflammatory cells are the potential mediating agents of UV-induced cytokines that leads to epidermal proliferation of keratinocytes and hyperplastic lesion.

The principle bioactive components of red cabbage are isothiocyanates, vitamins A, B, C, and anthocyanins. Anthocyanins, a natural pigment present in red cabbage, were having the most potent antioxidant properties of 150 flavonoids. They are water-soluble pigments it can be red, blue or purple depending on the pH. They are dominant antioxidants that have anti-inflammatory properties which help to protect cells. Along with the "substances that seem to be responsible for the biological activities of red cabbage, are polyphenols" (Hassimotto et al., 2010).

In this research, we mainly study the effect of antioxidant on skin aging. Thus, red cabbage (Brassica oleracea L. var. capitata f. rubra) is the antioxidant for this study. The antioxidant properties are directly measured against the thickness of the dermis of the Wistar male mice that have been exposed to UV-B with different concentrations such as 5%, 10%, and 20%. From the literature review, can be seen that the effect of purple cabbage ethanol cream extract on male Wistar mice with the exposure of UV-B have not been done for any research studies.

Based on the results given, there are differences in the thickness of the dermis according to the dose of ethanol red cabbage cream with the exposure of UV-B. Increase in the dosage shows apparent results and there is an increase in the thickness of the dermis. Based on earlier research, its proven that red cabbage has antioxidant and anti-inflammatory properties which affect the thickness of dermis in male Wistar mice.

Throughout this study, specific factors may affect the results of the experiment. Based on the results, the duration of red cabbage extract given is short thus may not produce results as expected.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total amount of samples (n)</th>
<th>Mean ± SD (µm)</th>
<th>Normality (Shapiro-Wilk)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (P1)</td>
<td>5</td>
<td>504.10 ± 129.40</td>
<td>0.739</td>
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<tr>
<td>Base cream (P2)</td>
<td>5</td>
<td>544.76 ± 100.67</td>
<td>0.659</td>
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<tr>
<td>5% ethanolic extract (P3)</td>
<td>5</td>
<td>547.43 ± 188.41</td>
<td>0.240</td>
<td>0.39</td>
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<tr>
<td>10% ethanolic extract (P4)</td>
<td>5</td>
<td>651.19 ± 189.18</td>
<td>0.021</td>
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</tr>
<tr>
<td>20% ethanolic extract (P5)</td>
<td>5</td>
<td>666.60 ± 143.15</td>
<td>0.146</td>
<td></td>
</tr>
</tbody>
</table>

*) statistically significant at P < 0.05; SD = Standard deviations; P = P-value

Figure 1. The microscopic histology preparations of the dermis skin layer using H&E staining (Light microscope; 10 x 40 magnification) (P1 = control group; P2 = Base cream; P3 = 5% ethanolic extract cream of red cabbage; P4 = 10% ethanolic extract cream of red cabbage; and P5 = 20% ethanolic extract cream of red cabbage)
CONCLUSION

Based on the results of the research that has been done, it can be concluded that red cabbage ethanol extract has no significant towards the thickness of the dermis in Wistar male mice. In order to get a significant result, more study have to be conducted.

RECOMMENDATION

There are a few suggestions that can be given to further this research:
1. The dose of 5%, 10% and 20% of red cabbage ethanol extract can be increased to identify the maximum dosage for this research study.
2. The duration of this research should be longer than 2 weeks to get a better and more significant results. It may take a longer duration to administer the extract into the skin of the mice.

REFERENCES