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The effect of red-cabbage (*Brassica oleracea L. var. capitata f. rubra*) ethanolic extract in cream preparation on dermis-elastic fibre thickness in male Wistar rats after ultraviolet-B rays exposure

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ABSTRACT

Background: Ultraviolet B (UV-B) rays exposure is a leading cause of sunburn, skin dryness and skin aging by influencing the dermal elastic fibers. This photoaging effect was caused by damage of fibrillin-1 as a microfibrillar network. This study aims to evaluate the effect of red-cabbage (*Brassica oleracea L. var. capitata f. rubra*) ethanolic extract in cream preparation on dermis-elastic fibers thickness in male Wistar rats after UV-B exposure.

Methods: A randomized post-test only control group design was conducted among 25 male Wistar rats 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/cm^2 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 a statistically significant difference of dermal-elastic fibers thickness among groups ($P < 0.05$). The mean of dermis-elastic fiber thickness was 6.125±0.376 µm in P5 group, followed by 4.075±0.266 µm in P4 group, and 3.114±0.290 µm, 2.109±0.420 µm, and 1.907±0.454 µm in P3, P2, and P1 group respectively.

Conclusion: Ethanolic extract of red-cabbage in a different dose of cream preparation have a statistically significant difference on the dermis-elastic fibers thickness after UV-B exposure

Keywords: dermis-elastic fiber, ethanolic extract, red cabbage, thickness

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INTRODUCTION

Skin human is divided into three primary layers, from inside to outside which subcutaneous fat layer, the dermis, and the epidermis. The dermis is the major component of the skin which is made up of collagen, elastin, and glycosaminoglycans, collectively termed as an extracellular matrix.[1] Tissue flexibility and extensibility have been essential requirements in the evolution of multicellular organisms. Elastic fibers are major insoluble extracellular matrix (ECM) assemblies that endow connective tissues with resilience, permitting long-range deformability and passive recoil without energy input.[2] These properties are critical to the function of arteries, which undergo repeated cycles of extension and recoil, and to lungs, skin and all other dynamic connective tissues. This elastic function complements collagen fibrils, which impart tensile strength in skin aging.[2]

Skin is exposed to a variety of environmental factors such as UV radiation that originated from the sun. UV-rays exposure is a major causative factor for age-related changes such as skin cancer development due to the anatomic location at the external boundary of the body related to photoaging. Photoaging of skin is characterized by wrinkles, brown spot, uneven pigmentation, laxity, and a leathery appearance. In contrast, chronologically aged skin that has been protected from the sun is thin and has reduced elasticity but in otherwise smooth and unblemished.[3]

Ultraviolet (UV) radiation is composed of UVA, UVB and UVC components based on wavelength. UVA is having the longest wavelength (315-400nm), followed by UVB as mid-range of wavelength (290-320 nm), and UVC as the shortest wavelength (100-280 nm). Furthermore, ambient sunlight is composed primarily of UVA (90%-95%) and UVB (5%-10%) energy, with most solar UVC absorbed by the ozone layer.[4] Reactive oxidative species (ROS) are produced by cells during normal

metabolic activities such as mitochondrial oxidative phosphorylation where its level varies based on UV exposure and levels of antioxidants. UV-B induces a variety of free radical and oxidative molecules, which because of their chemical reactivity alter the molecular structure and damage those proteins, nucleic acid, and lipids.[4] Thus, antioxidants enzymes mediate the removal of ROS, with different enzymes functioning in specific compartments. If the ROS is not being removed, then it may react with DNA and other cell signaling protein which impair their function and indirectly induce skin aging.[5]

The loss of elastic fiber integrity in response to solar UV exposure leads to a marked reduction of skin elasticity and manifests as skin wrinkles. A similar loss also occurs during intrinsic aging process, but it is generally associated with a progressive loss of elasticity. Loss of elasticity as a result of elastic fiber degradation is a major contributing factor in aging of connective tissues including the lungs, the cardiovascular system, and the dermis of the skin.[6] Photoaging of skin also reveals as a loss of fibrillin-rich microfibrils at the DEJ, which implies that fibrillin-1 is one of the first constituents of the microfibrillar network to be damaged by solar exposure. Fibrillin-1 can be considered as an early marker of photoaging.[6]

The –OH groups in phenolic compounds are thought a significant role in antioxidant activity. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties.[7] Phenolic compounds are known to be the major group with antioxidant capacity in Brassica crops. These compounds are able to scavenge reactive oxygen species due to their electron donating properties. Phenols and anthocyanins contained may protect cells from free radical damage by donating electrons.[7] Thus the phenol content and the ability of anthocyanins in purple cabbage can prevent premature aging of the skin by protecting the skin from the formation of ROS by exposure to UV induction. The most widespread and diverse group of polyphenols in Brassica species are flavonoids and hydroxycinnamic acids. In many in vitro studies, phenolic compounds demonstrated higher antioxidant activity (AA) than other antioxidants, such as vitamins and carotenoids.[8]

METHODS

A randomized post-test only control group design was conducted among 25 male Wistar rats during 2 weeks at Laboratory Animal Unit, Department of Pharmacology, Faculty of Medicine, Udayana University. They were divided into 5 different groups namely: positive control group (P1), negative control group (P2) with base cream, Treatment group 1 (P3) with 5% ethanolic extract cream of red-cabbage ethanol, Treatment group 2 (P4) with 10% ethanolic extract cream of 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 /cm^2 prior study until 2 weeks. 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 elastic-fiber was assessed at Department of Histology, Faculty of Medicine, Udayana University using Image Raster software.

The data obtained was statically analyzed using 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 regarding the thickness of dermis elastic-fiber was shown in Figure 1 below. Skin tissue was stained by Haematoxylin and Eosin (HE) and observed by using a light microscope with 10 x 40 magnification. Macroscopically, the thickest of dermis elastic-fiber 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 (6.125±0.376 μm), followed by P4 (4.075±0.266 μm), P3 (3.114±0.290 μm), P2 (2.109±0.420 μm), and P1 (1.907±0.454 μm) (Table 1).

Data of the dermis-elastic fiber thickness in each group was tested for normality and homogeneity by using Shapiro-Wilk and Levene test. The results show that the elastic fibers of dermis data were normally distributed with $P > 0.05$ (Table 1).

The result was also analyzed by using one way ANOVA method which showed that there was a significant difference among groups ($P < 0.05$). Multivariate analysis using LSD was also found a significant difference between groups ($P < 0.05$) (Table 1).

DISCUSSION

This study proved that the cream extract of purple cabbage ethanol either cream 5%, 10%, or 20% were able to protect the effects of acute UV exposure. The repeated UV exposure at sub-erythemal doses elicits a gradual increase in the expression of matrix metalloproteinases (MMPs) with elastase activity

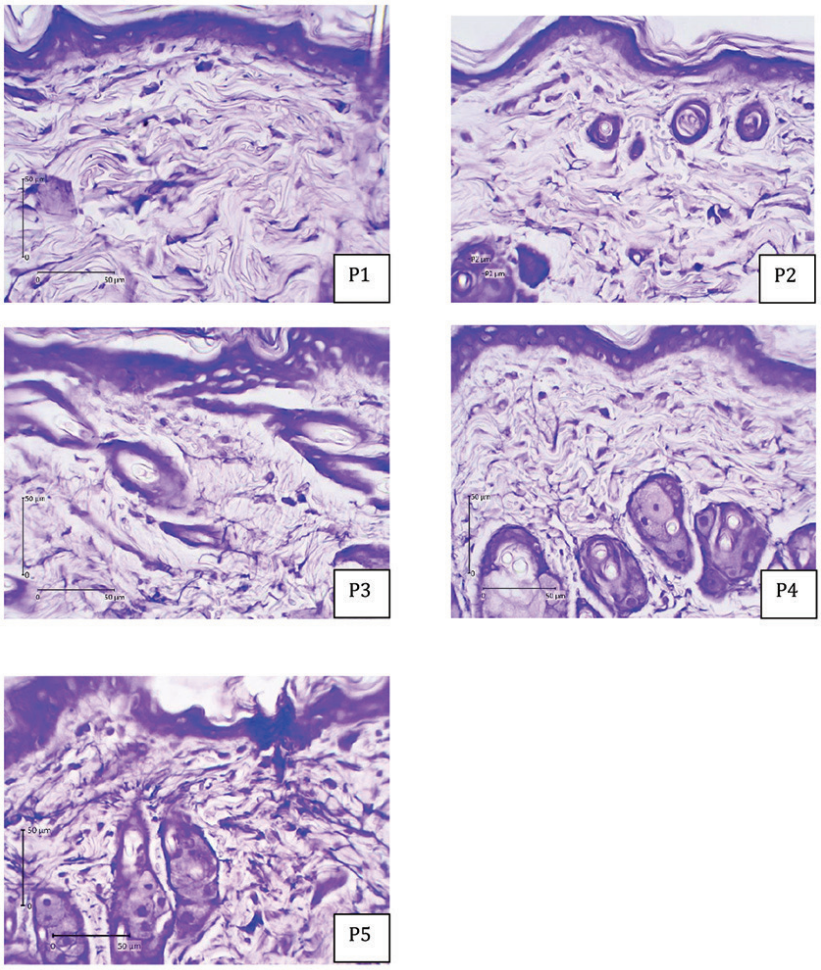

Figure 1. The microscopic histology preparations using H&E stain of the dermis-elastic fibers thickness (Light microscope; 10 x 40 magnification) (P1 = control group; P2 = Base cream; P3 = 5% ethanolic extract cream of red cabbage; P4 = 10% ethanolic extract cream of red cabbage; and P5 = 20% ethanolic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

Table 1. The mean of dermis-elastic fiber in different groups of male Wistar rats after UV-B exposure

Groups	Total amount of samples (n)	Mean ± SD (µm)	Normality (Shapiro-Wilk)	Homogeneity	P
Control (P1)	5	1.907±0.454	0.945		
Base cream (P2)	5	2.109±0.420	0.649		
5% ethanolic extract (P3)	5	3.114±0.290	0.241	0.673	0.000*
10% ethanolic extract (P4)	5	4.075±0.266	0.875		
20% ethanolic extract (P5)	5	6.125±0.376	0.829		

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

in the dermis. The enhanced activity of elastases would affect the elastic fiber network, where fibroblasts are anchored via their plasma membrane to the surrounding dermal connective matrix tissue. Resulting in their curling even in the absence of cutaneous inflammation during which neutrophils and macrophages frequently infiltrate the dermis.[9] Thus, the tortuous deformation of elastic fibers is strongly indicative of the pin-point disconnection through the elastic fiber network by enhanced elastase activity, which is produced and expressed on the plasma membrane of dermal fibroblasts but not by neutrophils or macrophages.[9]

Elastic fibers of dermis are composed of elastin core and microfibrillar scaffold, which in adult tissues comprises mainly the glycoprotein and fibrillin. This network of fibers imbues skin with elasticity. Elastic fibers are required to maintain their elastic function for a lifetime. However, various enzymes, such as matrix metalloproteinases (MMPs), are able to cleave elastic fibers molecules.[10] The preventive effect of the inhibitor for skin fibroblast-derived elastase on wrinkle formation was also corroborated by animal and human clinical studies using an extract of purple cabbage, which was found to be capable of inhibiting skin fibroblast-derived elastase but not neutrophil elastase.[10]

The photoaged skin loss of fibrillin-rich microfibrils at DEJ, which implies that fibrillin-1 is one of the first constituents of the microfibrillar network to be damaged by solar exposure. The previous study strongly indicate that enhanced activity of dermal fibroblast-derived elastase plays an essential role in deteriorating the elastic fiber network, resulting in the deficiency of skin elastic properties.[9]

Similar studies had also been conducted by Gek Ayu Krismayogi, from the research showed that the presence of polyphenol compounds in the purple cabbage which has rich antioxidant activity is able to decrease the erythema of the treatment group more quickly than the control group.[11] Besides, in accordance with several studies both in animals and humans that proved the benefit of antioxidants against UVR effect.

Data from the treatment of the placebo group, 5% 10% and 20% concentration of ethanol red cabbage showed the mean of the dermis-elastic fibers in male Wistar rats increased compared to the control group. As the higher the cream extract of purple cabbage ethanol is the best result can be achieved. Thus, the results indicated that purple cabbage extracts have an ability to inhibit skin fibroblast-derived elastase proved to be effective as anti-wrinkling agents, confirming the essential role of elastase in UVB-induced wrinkle formation.

CONCLUSION

The ethanolic extract cream of red-cabbage (*Brassica oleracea L. var. capitata f. rubra*) in different dosage have a beneficial effect on the thickness of dermis-elastic fibers among male Wistar rats after exposed to UV-B rays. Further studies are needed to assess the minimum dosage, mechanism, side effects, clinical benefits, and human studies.

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REFERENCES

1. Borumand M, Sibilla S. Daily consumption of the collagen supplement reduces visible signs of aging. Clin Interv Aging. 2014; 9:1747-1758.
2. Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. J Cell Sci. 2002; 115(Pt 14):2817-28.
3. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med. 1997; 337(20):1419-28.
4. Amaro-Ortiz A, Yan B, D'Orazio J. Ultraviolet radiation, aging and the skin: prevention of damage by topical cAMP manipulation. Molecules. 2014; 19(5):6202-6219.
5. Poljsak B, Dahmane R. Free radicals and extrinsic skin aging. Dermatol Res Pract. 2012; 10:1155-1352.
6. Langton AK, Sherratt MJ, Griffiths CEM, Watson RE. A new wrinkle on old skin: the role of elastic fibres in skin ageing. Int J Cosmet Sci. 2010; 32(5):330-9.
7. Ahmed MF, Rao AS, Ahemad SR, Ibrahim M. Phytochemical Studies and Antioxidant Activities of *Brassica oleracea L. var. Capitata*. Int J Pharm Pharm Sci, Vol 4, 2012; 3:374-378.
8. Sotelo T, Cartea ME, Velasco P, Soengas P. Identification of Antioxidant Capacity Related QTLs in *Brassica olerace*. PLoS One. 2014; 9(9): e107290.
9. Imokawa G, Ishida K. Biological mechanisms underlying the ultraviolet radiation-induced formation of skin wrinkling and sagging I: reduced skin elasticity, highly associated with enhanced dermal elastase activity, triggers wrinkling and sagging. Int J Mol Sci. 2015; 16(4):7753-75
10. Bhattacharyya TK, Thomas JR. Histomorphologic changes in aging skin observations in the CBA mouse model. Arch Facial Plast Surg. 2004; 6(1):21-5.
11. Krismayogi GA, Ratnayanti IGAD, Linawati NM, Wiryawan IGNS, Sugiritama IW, Wahyuniari IAI, Arijana IGKN. Purple Cabbage Extract Cream Effect on Erythema Score of Male Wistar Rats Back Skin Exposed to UV-B Radiation. Biomedical and Pharmacology Journal. 2018; 11(1):345-351