

Topical administration of broccoli flower (*brassica oleracea l. Var italica plenck*) extract cream 10% prevented increased of melanin in UVB exposed guinea pig (*cavia porcellus*)

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Introduction: Skin aging is primarily caused by increased free radicals and decreased cellular anti-oxidant system in keratinocyte. Broccoli flower contains several important antioxidant molecules that can inhibit aging process of the skin. Therefore, the aim of this research is to prove the effectivity of administration broccoli flower extract cream on inhibiting the elevation of melanin level on guinea pig (*Cavia porcellus*) exposed with UV-B.

Methods: An animal experiment with *post-test only controls group design* was conducted using 36 guinea pigs which were divided into 2 groups. The control group was treated with placebo cream while treatment group was treated with 10%

broccoli flower extract cream. All groups were exposed with UV-B with dose at 390 mJ/cm² for 2 weeks and biopsy was taken to examine the level of melanin.

Results: The results showed that the average amount of melanin expression in the control group (P0) was 21.48 ± 1.48% while it was 1.32 ± 0.85% in treatment group (P1). Independent T-test analysis showed that the difference was statistically significant (t = 33.95; p = 0.001).

Conclusion: It can be concluded that the topical administration of broccoli flower extract cream prevented increase in skin melanin in UV-B exposed guinea pig.

Keywords: 10% broccoli flowers extract cream, melanin, UV-B ray, guinea pig

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INTRODUCTION

Aging or aging process is a physiological process that will occur in all living things which includes all organs of the body. The intrinsic aging process is a physiological aging process that takes place naturally due to various factors in the body itself such as genetic, hormonal, and racial. Extrinsic aging process occurs due to various factors from external environment such as sunlight / ultraviolet light, air humidity, temperature, cigarette smoke, and various other external factors that can accelerate aging of the skin (premature aging). Various efforts can be made to prevent or avoid factors that accelerate the process.^{1,2}

UV light exposure can induce hydrogen peroxide and superoxide anions formation which produced by melanin and cause mutations in melanocytes and other cells.³ The process of melanin formation in melanosomes begins with hydrosylation of L tyrosine to L-dopa and oxidation of L-dopa to dopakuinone, both of these processes require activation of the enzyme tyrosinase. Dopakuinone will be polymerized spontaneously to form melanin. Tyrosinase enzyme will work directly during UV stimulation. In this process, there is an increase in tyrosinase enzyme activity and melanin formation,

an increase in the number of melanocyte cells and an increase in the distribution of melanin to keratinocytes, this pigmentation begins 2-3 days after exposure and lasts for 10-14 days.¹

Broccoli flower (*Brassica oleracea l. var. Italica plenck*) is a type of cabbage plant that is known to be rich in antioxidants. The content of broccoli includes sulforafan (glycosides), quercetin and flavonoids (anthocyanins, polyphenols), vitamin A, vitamin C, vitamin E, vitamin BI, vitamin B2, betacarotene, iberine and cyanohydroxy butene.⁴ Previous phytochemical screening studies showed that broccoli contained alkaloids, saponins, steroids/triterpenoides and extracelleyl acetate fractions containing glycosides, sulforafants, flavonoids and vitamin C.⁵ Broccoli flowers contain polyphenols on a dry base of 463.15 ± mg TAE and a wet base of 39.48 ± 0.20 mg TAE (Mandarini, 2014). While in the preliminary study at the Faculty of Agricultural Technology, Udayana University broccoli contained polyphenols at 792.63 mg / 100g GAE on a dry basis and 99.94 mg / 100g GAE on a wet basis.⁵ In addition, it contains vitamin C at 55.90mg /100g bb. Vitamin C is one of the compounds that can be used as antioxidant, which is one of the secondary antioxidants that has the ability to capture free

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radicals and prevent chain reactions.⁶

In one of the study, topical 10% broccoli flower extract cream was proved to be the most effective in suppressing melanogenesis in guinea pigs exposed to UVB rays. Taking this into account, there is an indication that polyphenols (flavonoids) which are widely found in broccoli flower extracts can inhibit premature aging of the skin by inhibiting tyrosinase in guinea pigs that are exposed to UVB light. Therefore, it is necessary to prove this concept in an experimental research.

METHODS

This study was an experimental study with a *randomized post test only control group* design. The study used two groups with each group comprised of 18 female guinea pigs in each group. Control group 0 (P0) was treated by placebo cream which applied 20 minutes before UV-B exposure. Treatment group 1 was treated by 10% broccoli mixture cream extract that applied 20 minutes before UV-B exposure. All animals were exposed to UV-B 3 times a week with a total dose of 390 mJ/cm² for 2 weeks. The same cream was re-applied to each group after 48 hours of rest to eliminate the acute effects of UV-B radiation. Skin tissue biopsy and histopathological examination with Masson-Fontana staining were used to calculate the amount of melanin. All animal experiments were carried out at the Integrated Biomedical Laboratory Unit FK Udayana Laboratory Animal Unit Pharmacology Section while broccoli flower extract cream production was conducted at the Pharmacology section of Faculty of Medicine Udayana University. Histology examination and printing of Masson Fontana skin tissue were carried out at the Histology Laboratory of Faculty of Medicine Udayana University.

RESULTS

36 guinea pigs (*Cavia porcellus*) aged 3-4 months, with a body weight of 300-350 grams were used as a sample which were divided into control and treatment groups as stated above. The mean melanin concentration in the control group was 21.48± 1.48% with a range 17.87-26.41. Meanwhile, the average concentration of melanin in the treatment group was 1.32±0.85% with a range between 0.64-2.21. According to Shapiro-Wilk and Levene's test, all of the data were normally distributed and homogenous ($p > 0.05$).

After two weeks of treatment, the Guinea Pig's back skin tissue was biopsied for histopathological examination. Melanin appeared black in Masson-Fontana staining. The histological presentation of melanin in the guinea pig epidermal tissue is showed in Figure 1. Control group appeared to have densely packed melanin in groups which congregate at the the basal area and the entire upper layer of the epidermis. In contrast, melanin in the treatment group appeared to be sparser with smaller group and evenly spread in the upper layer of the epidermis.

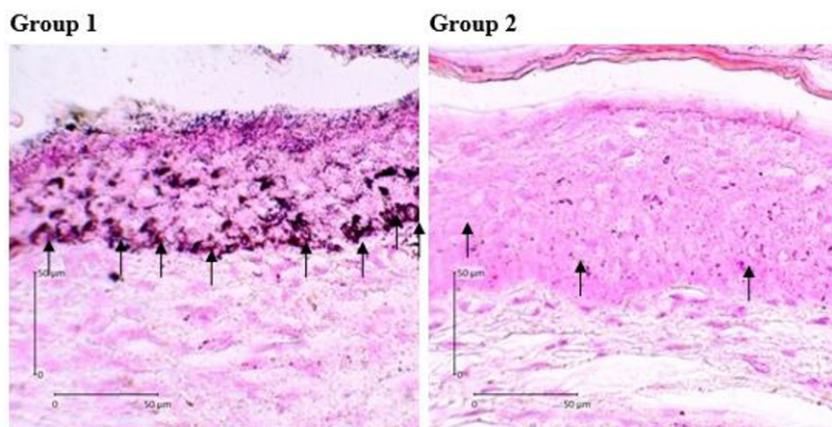
Table 1 shows the comparison between P0 and P1 at the end of the experiment. Control group appeared to have higher melanin concentration at 21.48 ± 1.48% while melanin concentration was only at 1.32 ± 0.85% in the treatment group. Independent T-test analysis showed that the difference was statistically significant ($t = 33.95$; $p = 0.001$).

Table 1. Difference in Average Amount of Melanin Between Groups After Given Exposure to Ultraviolet B Light and 10% Broccoli Flower Extract Cream

Subject	N	Melanin Average	SB	t	p
Control	18	21,48	1,48	33,95	0,001
Treatment	18	1,32	0,85		

DISCUSSION

This study showed evidence that broccoli extract can be used to prevent skin aging. Although not assessed in this study, it is believed that the anti-oxidant properties of compounds in broccoli extract prevent the formation of free-radical in the presence of UVB exposure. Several important anti-oxidant compounds in broccoli flowers that can inhibit melanogenesis as will be described in the following sections. One of those compounds is Flavonoids, a group of phenol compounds



Picture 1. Picture of Guinea Pig Skin's Melanin with Masson-Fontana Staining

that function as antioxidants. Flavonoids are able to inhibit the oxidation reaction through the mechanism of counteracting free radicals by donating one unpaired electron. Flavonoids are strong inhibitors of lipid peroxidation and are also able to inhibit the activity of lipo-oxygenase and cyclooxygenase enzymes.⁶

Other than Flavonoids, Vitamin C or ascorbic acid is also one of the potent antioxidants and has been shown to minimize erythema and sunburn after UVB exposure. Vitamin C consists of 6 lactone carbon chains that are synthesized from glucose in the liver of mammals except humans, because humans do not have the enzyme gluconolactone oxidase which can synthesize ascorbic acid from glucose.^{1,7}

The third content is tannins, which are natural phenolic acids containing two different carbon chains cinnamic hydroxy acid and hydroxybenzoate.⁸ Flavonoids come from a large group of low molecular weight polyphenols and benzo- γ -pyrone derivatives. Tannins are antioxidants and also have the ability to act as anti-tyrosinase because they inhibit the biosynthesis process of melanin so that increased production of melanin does not occur after UVB exposure.⁹

Enzymatic and non-enzymatic antioxidants will interact to provide a better protection against oxidative stress including vitamin E, coenzyme Q10, ascorbate, carotenoids, superoxide dismutase, catalase, and glutathion peroxidase. However, due to excessive exposure to ultra violet, resulting in depletion of the antioxidant supply, a state of oxidative stress is formed. For this reason, topical antioxidants are also needed to increase the antioxidant reserves of the skin. In skin malignancies such as basal cell carcinoma, low levels of carotenoids are found, so it is estimated that these antioxidants are very important in skin defense against UV radiation and photocarcinogenesis.¹⁰

CONCLUSION

According to the result of this study, 10% broccoli flower extract cream application prevents an increase in melanogenesis in the skin of guinea pigs (*Cavia porcellus*) exposed to Ultraviolet B rays.

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 407/KE-PH-Lit-2/VII/2019

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