ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2022, Volume 11, Number 2: 527-530
P-ISSN.2089-1180, E-ISSN: 2302-2914

The potential of carrot extract as a sunscreen to prevent apoptosis in white mice (*Mus musculus*) fibroblast cell cultures exposed to UVB light

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Received: 2022-04-11
Accepted: 2022-06-02
Published: 2022-06-28

ABSTRACT

Introduction: UVB rays are one of the main external factors that play a role in the aging process. Apoptosis of fibroblasts affects skin aging, and the decline in fibroblasts is also caused by the reduced number and ability of growth factors and other hormones. Sunscreen has been used for a long time to protect the skin from the harmful effects of solar radiation, especially UVB rays. Antioxidant activity such as β-carotene and vitamin E from carrots can counteract free radicals and oxidative stress caused by UV radiation. This study aims to test whether in vitro administration of carrot extract can be used as a sunscreen to prevent apoptosis in mouse fibroblast cell cultures exposed to UVB light.

Method: This research is a laboratory experimental study, using a Randomized Post-test Only Control Group Design. The research sample was fibroblast cell culture derived from the back skin of white mice (*Mus Musculus*) isolated with Collagenase 1 (primary culture). Observation of fibroblast apoptosis using propidium iodide staining. Data were analyzed using Shapiro-Wilk test, Levene's test, One Way Anova and continued with LSD test. The confidence level in this study is 95%.

Result: There was a significant difference between the control group and the carrot extract treatment group, but no significant difference was found between the SPF15 group and the carrot group ($p>0.01$).

Conclusion: Carrot extract can provide a protective effect on the skin (fibroblasts) and prevent apoptosis.

Keywords: Aging, apoptosis, carrots, fibroblasts, UVB rays.
Cite This Article: Satriyasa, B.K., Widianti, I.G.A., Manuaba, I.B.G.F. 2022. The potential of carrot extract as a sunscreen to prevent apoptosis in white mice (*Mus musculus*) fibroblast cell cultures exposed to UVB light. *Bali Medical Journal* 11(2): 527-530. DOI: 10.15562/bmj.v11i2.3460

INTRODUCTION

There are several factors that contribute in the aging process, which can be divided into two groups, namely internal factors and external factors. External factors include unhealthy diet, unhealthy lifestyle, wrong habits, environmental pollution, radiation, UV rays, cigarette smoke, and stress.[1,2] UVB rays are the smallest fraction of total UV radiation (approximately 10% of total UV) but is the main cause of erythema (sunburn), browning of the skin (tanning), carcinogenesis due to sunlight (photocarcinogenesis) and premature aging due to sunlight (photoaging).[3,4] Various markers to assess aging process has been reported. Fibroblasts are the most abundant cells in connective tissue. The process of fibroblast apoptosis will affect skin aging, the decrease in fibroblasts is also caused by the reduced number and ability of growth factors and hormones that will help prevent the aging process.[5]

Sunscreen has been used for a long time to protect the skin from the harmful effects of solar radiation, especially UVB rays.[4] However, the sunscreens that have been circulating are relatively expensive, so they are not widely consumed by people with low economic class.[6] Carrots (*Daucus carota L.*) are rich in antioxidant compounds such as β-carotene and vitamin E.[7] β-carotene functions as a free radical scavenging agent, anti-mutagenic, chemopreventive, photoprotective and immunoenhancing agent.[8] This causes β-carotene has the potential as a sunscreen. Carrots also contain vitamin E in the form of tocopherols which can block the active compounds from UV exposure.[9] Carrot extract has also been shown to inhibit the tyrosinase enzyme which plays a role in the formation of the melanin pigment.[10] Consequently, carrots have the potential as a natural sunscreen. This study aims to test whether in vitro administration of carrot extract can be used as a sunscreen to prevent apoptosis in mouse fibroblast cell cultures exposed to UV light.

MATERIAL AND METHODS

This research is a laboratory experimental study, using a Randomized Post-test Only Control Group Design. This research was conducted in the integrated laboratory of the Faculty of Medicine, Universitas Udayana. The research sample was fibroblast cell culture from the back skin of 2 white mice (*Mus musculus)* isolated with Collagenase 1 (primary culture). The research sample was divided into 3 groups, namely the control group which was only given 30 minutes of UVB irradiation, treatment group 1 (the group that was added to parasol with SPF 15 and given UVB irradiation for 30 minutes), and the treatment group 2 (the group was added with carrot extract and given UVB irradiation for 30 minutes). Each group contains 10 samples of treatment

repetition. Carrot extract was processed in a blender, centrifuged, and then washed with acetone, hexane, and aquadest. UVB irradiation using the Cambridge UVITEC apparatus. Observation of fibroblast cells using propidium iodide staining, apoptotic cells are yellow-green (fluorescen:12dUTP) on a red background. Observations were made in every 100 cells in each visual field, in 3 fields of view. Data were analyzed using Shapiro-Wilk test, Levene's test, One Way Anova and continued with LSD test. The confidence level in this study is 95%.

RESULT

The descriptive analysis results of the data on the percentage of apoptotic cells in the three groups are displayed in Table 1. The percentage of apoptotic cells in each group was tested for normality using the Shapiro-Wilk test. The results show that the data is normally distributed (p> 0.05), which is presented in Table 2. Homogeneity analysis of the data with Levene's test has shown that the data is homogeneous (p>0.05) (Table 3). Comparability analysis aims to compare the mean percentage of apoptotic cells between groups. The significance analysis was tested by ANOVA test because the distribution of the data was normal and homogeneous. The results of the comparative analysis are presented in Table 4 and the LSD test is described in Table 5.

ANOVA test shows there was a significant difference on the mean of apoptotic cells between groups (p <0.001) (Table 4). LSD test has shown that was a significant difference between the control group and the carrot extract treatment group, but no significant difference was found between the SPF15 group and the carrot group (p>0.01) (Table 5). Based on these statistical results, it can be concluded that the administration of carrot extract was as effective as the administration of SPF15. The difference in the percentage of apoptotic cells between groups can also be seen in Figure 1.

DISCUSSION

Carrots are known for their bioactive components which are rich in lipophilic (carotenoid) and hydrophilic (phenolic components) antioxidants. The components in carrots include phenolics, β-carotene, ascorbic acid, tocopherols, and various vitamins ranging from vitamins A, B, C and E.[11] β- carotene functions as a free radical scavenging agent, single oxygen scavenger and has anti-mutagenic, chemopreventive, photoprotective and immunoenhancing effects.[8] This causes β-carotene has the potential as a sunscreen. The β-carotene contained in carrot extract has a relatively low SPF, which is <4 in the UVB spectrum range.[12]

This study found that was a significant difference on the apoptotic cells between the control group and the carrot extract treatment group (mean difference: 37.2; 95% CI: 31.66-42.74; p<0.001), but no significant difference was found between the SPF15 group and the carrot group (p>0.01). Based on previous research, the combination of using β-carotene lotion and sunscreen has been shown to be effective and safe in treating melasma.[13] Topical application of β-carotene can neutralize free radicals produced on the skin surface exposed to stress factors, such as infrared irradiation.[14] Carrots contain the main phenolic compounds in the form of hydroxycinnamic acids and their derivatives. The phenolic content in

Table 1. The descriptive analysis results of the data on the mean of apoptotic cells.

Groups	Mean	Standard Deviation
Control	65.80	5.827
Treatment with SPF 15	23.50	4.696
Treatment with carrot extract	28.60	7.291

Table 2. The normality test results of the apoptotic cell data between groups.

Groups	Shapiro-Wilk	
	Statistic	p Value
Control	0.143	0.909
Treatment with SPF 15	0.142	0.967
Treatment with carrot extract	0.178	0.668

Notes: p>0.05 means that the data is normally distributed

Table 3. The results of the homogeneity on the mean of apoptotic cell data between groups.

	Levene Statistic	p Value
Based on Mean	0.886	0.424
Based on Median	0.929	0.407
Based on Median and with adjusted	0.929	0.409
Based on trimmed mean	0.803	0.421

Notes: p>0.05 means the data group comes from a homogeneous population

Table 4. ANOVA test results on the mean of apoptotic cells between groups.

	N	Mean (SB)	p
Control	10	65.80 (5.827)	
Treatment with SPF 15	10	23.50 (4.696)	<0.001
Treatment with carrot extract	10	28.60 (7.291)	

Table 5. LSD test results on the apoptotic cells between groups.

	Mean Difference	95% CI	p
Control vs SPF-15	42.3	36.76-47.84	<0.001
Control vs Carrot Extract	37.2	31.66-42.74	<0.001
SPF-15 vs Carrot Extract	5.1	-0.44 – 10.64	0.069

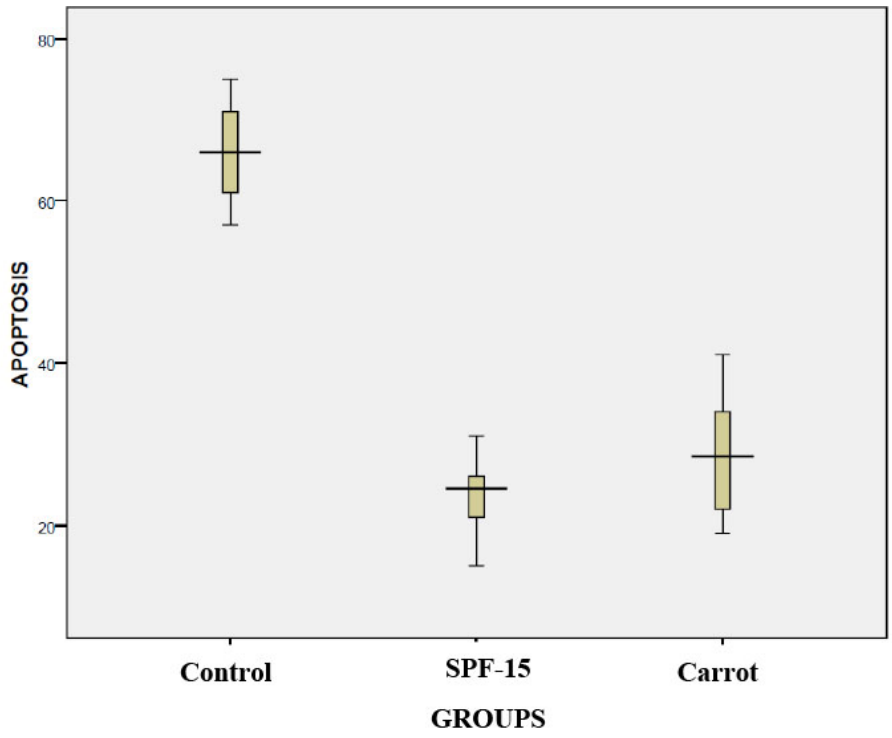

Figure 1. Differences in the percentage of apoptotic cells between groups.

carrots decreases in the following order: skin – phloem – xylem. Antioxidant and radical scavenging activity also decreases following the order of phenolic levels in the tissue. Chlorogenic acids are the most common hydroxycinnamic acids found in carrots, accounting for 42.2–61.8% of the total phenolic compounds.[15] Chlorogenic acids are reported to be the most abundant phenolic acids in plant extracts and are the most active antioxidants. Phenolic compounds in various plants can protect cells from oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (ROS).[16] Carrot extract contains phenolic compounds and vitamins A and E which can provide better skin protection from sun radiation. Vitamin E also functions as an antioxidant and has photoprotective activity against UV rays. Vitamin E in the form of -tocopherol produces maximum absorbance at UVB (λmax = 292 nm). α-tocopherol can provide protection from UV-induced photodamage through its ability as a sunscreen. Endogenous α-tocopherol can prevent UV-induced lipid peroxidation by acting as a chain-breaking antioxidant in the skin. Topical α-tocopherol is not effective in penetrating the epidermis and thus provides protection as a sunscreen.[17] Vitamin E can block UV rays from interacting with active ingredients, thereby preventing photodegradation of active ingredients in sunscreens.[9]

The limitation of this study is that it applied the in-vitro method, so further research is needed to prove the effectiveness of carrot extract as a sunscreen with the in vivo method, which is then followed up with clinical trials in humans.

CONCLUSION

From the results of the study, it can be concluded that carrot extract can provide a protective effect on the skin (fibroblasts) and prevent apoptosis.

CONFLICT OF INTEREST

There is no conflict of interest regarding this article.

FUNDING

This research did not receive funding from any party.

ETHICAL CONSIDERATION

The methodology of this study was approved by the Ethical Clearance Commission (No: 09/07/FKUNUD/2021).

AUTHOR CONTRIBUTION

All authors contributed equally to this study.

REFERENCES

1. Rabe JH, Mamelak AJ, McElgunn PJ, Morison WL, Sauder DN. Photoaging: mechanism and repair. *Journal of the American Academy of Dermatology*. 2006 ;55(1):1-9.
2. Pangkahila W. Anti Aging Medicine:Memperlambat Penuaan, Meningkatkan Kualitas Hidup. 2nd Ed. Jakarta: Kompas; 2011.
3. Stanfield J. UVA Protection: An Update. Suncare Research Laboratories, *LLC*. 2006:4-9.
4. Ikhtiyati N, Etnawati K, Widodo YW. The prevention of the occurrence of ultraviolet B (UVB) induced hypoxanthine guanidine phosphoribosyl transferase (HGPRT) mutant cells by several commercial suncreens – an *in vitro* study. *Berkala Ilmu Kedokteran*. 1998;30(3):139-43.
5. Mescher AL. Junqueira's Basic Histology. Twelfth Edition. New York: The McGraw-Hill Companies Inc. 2010;173-185.
6. Cho JW, Park K, Kweon GR, Jang BC, Baek WK, Suh MH, *et al*. Curcumin inhibits the expression of COX-2 in UVB-irradiated human keratinocytes (HaCaT) by inhibiting activation of AP-1: p38 MAP kinase and JNK as potential upstream targets. *Exp & Mol Med*. 2005;37(3):186-92.
7. Styawan AA, Hidayati N, Susanti P. Penetapan kadar β-karoten pada wortel (*Daucus carota L.*) mentah dan wortel rebus dengan spektrofotometri visibel. *Jurnal Farmasi Sains dan Praktis*. 2019;5(1):7-13.
8. Bayerl C. Beta-carotene in dermatology: does it help?. *Acta Dermatovenerol Alp Panonica Adriat*. 2008;17(4):160-2.
9. Probowati GA. Pengaruh vitamin E dan paparan sinar uv terhadap efektivitas in vitro krim tabir surya avobenzone dan octyl methoxycinnamate [skripsi]. Universitas Jember; 2015.
10. Hasrawati H. 2019. Uji aktivitas inhibitor tirosinase ekstrak n-heksan umbi wortel (*Ducus carrota L.*) [skripsi]. Universitas Islam Negeri Alauddin Makassar; 2019.
11. Salyati AR. Skrining fitokimia ekstrak wortel (*Daucus carota L.*) menggunakan agitated thin film evaporator bertekanan vacuum [skripsi]. Universitas Diponegoro; 2018.
12. Kar HK. Efficacy of beta-carotene topical application in melasma: an open clinical trial. *Indian Journal of Dermatology, Venereology, and Leprology*. 2002;68(6):320.

13. Darvin ME, Fluhr JW, Meinke MC, Zastrow L, Sterry W, Lademann J. Topical beta- carotene protects against infra-red-light–induced free radicals. *Experimental dermatology*. 2011;20(2):125-9.
14. Suman M, Kumari K. A study on sensory evaluation, beta-carotene retention and shelf-life of dehydrated carrot products. *J Food Sci Technol*. 2002;39:677–81.
15. Larson RA. The antioxidants of higher plants. *Phytochemistry*. 1988;27(4):969-78.
16. Krol ES, Kramer-Stickland KA, Liebler DC. Photoprotective actions of topically applied vitamin E. *Drug Metabolism Reviews*. 2000;32(3-4):413-20.
17. Shaath NA. Sunscreen regulation and commercial development third edition. New York U.S.A: *Alpha Research and Development White Plains*. 2005;55:34-40.

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