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Administration of Arabika Coffee Leaf Extract (*Coffea Arabica*) Reduces Tumor Necrosis Factor Alpha (TNF-α) and Sunburn Cell Levels in Male Wistar Rats (*Rattus norvegicus*) Exposed to Ultraviolet-B Rays

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ABSTRACT

Background: Ultraviolet (UV) radiation has acute and chronic impacts on the skin. However, UVB exposure can be prevented with available natural ingredients, especially those containing antioxidants. One of the natural ingredients that contains active compounds as antioxidants is arabica coffee leaves, which contain flavonoids that have antioxidant effects. ***Methods:*** This study was an experimental research with a post-test-only control group design, using 30 male Wistar rats (Rattus norvegicus) which were divided into 5 groups; the control group (P0), treatment group 1 (P1) were given placebo, while group 2 (P2), group 3 (P3) and group 4 (P4) was given UVB rays and given ethanol extract cream from Arabica coffee leaves at a dose of 2,5%, 5%, and 10% respectively. ***Results:*** The average TNF- α levels in group P0, P1, P2, P3, and P4 were 97,88±1,19 ng/mL, 162,41±1,87 ng/mL, 138,04±1,03 g/mL, 124,71±2,65 ng/mL and 107,18±1,52 ng/mL. The average sunburn cells in P0, P1, P2, P3, and P4 were 4,0±0,13, 4,07±0,27, 2,60±0,25, 1,67±0,15 and 1,33±0,12 per field view. The administration of 10% Arabica coffee leaf extract cream was the most effective in reducing TNF-α levels by 34.01% ($p<0.001$) and has the lowest sunburn cells per field view (1,33±0,12 cell/field view; $p < 0,001$). ***Conclusion:*** Administration of ethanol extract cream from Arabica coffee leaves (Coffea arabica) can reduce TNF-α levels and the number of sunburn cells in the skin of male Wistar rats (Rattus norvegicus) exposed to UVB radiation.

Keywords: Arabica coffee leaf, epidermis, TNF-A, sunburn cell

INTRODUCTION

Skin photoaging is caused by extrinsic and intrinsic factors. Extrinsic factors can be ultraviolet (UV) radiation, infrared radiation, and environmental carcinogens such as chronic sun exposure, air pollution, smoking, alcohol, and poor nutrition, while intrinsic factors can be genetic, cell metabolism, and hormonal.[1,2] Excessive exposure to ultraviolet light causes the accumulation of ROS resulting in TNF-α, a pro-inflammatory receptor on the surface of skin cells, becoming active. High levels of TNF-α indicate an active inflammatory process, causing the skin to appear reddish and sunburn.[3]

The use of cosmetic products to prevent photoaging is increasing along with technological developments and individual awareness to provide external protection against UV with sunscreen formulations that have high antioxidant substances.[4] However, the use of cosmetic products made from these chemicals causes many side effects, such as skin irritation, black spots, and even skin cancer with long-term use. Therefore, natural products really need to be researched and developed for dermatological purposes.

One of the natural ingredients in Indonesia which has been proven to contain active compounds as antioxidants is Arabica coffee leaves (*Coffea arabica*). The flavonoid content is able to inhibit the production of pro-inflammatory cytokines such as IL-1 beta, IL-2, IL-3, IL-6, IFN-gamma, TNF-alpha, and chemokines, where flavonoids work by inhibiting cyclooxygenase or lipoxygenase and inhibiting leukocyte accumulation the in areas of inflammation as an anti-inflammatory agent.[5,6]

After exposure to UV B, mast cells in the upper layer of the dermis release pro-inflammatory cytokine mediators and play a role in the mechanism of sunburn by releasing vasoactive substances that induce vasodilation and synthesize the formation of TNF-α. This TNF-α contributes to the regulation of apoptosis by releasing Inducible Nitric Oxide Synthase (iNOS) in macrophages.[7]

Based on this, the researchers wanted to know the efficacy of administering ethanol extract cream from Arabica coffee leaves (*Coffea arabica*) as an anti-inflammatory in reducing TNF-α levels and the number of sunburn cells in the epidermis of male Wistar rats (*Rattus norvegicus*) exposed to UV B radiation.

METHODS

This research is an experimental post-test-only control group design study, which was carried out in the period September 2023 to January 2024. The population of this study were male Wistar rats, aged 12-14 weeks, weighing 100-200 grams.

In this study, 30 samples will be divided into 5 groups, with the number of samples per group being 5 mice. Group 1 (P0) is a control group without treatment. Group 2 (P1) was given a placebo cream. Group 3 (P2) was given 2.5% coffee leaf ethanol extract cream. Group 4 (P3) received 5% coffee leaf ethanol extract cream, and group 5 (P4) received 10% coffee leaf ethanol extract cream. All groups received exposure to UV B light 3 times a week, for 4 weeks. Coffee leaf ethanol extract cream was given 30 minutes before exposure to UV B light. In the fourth week, the back skin tissue of experimental animals exposed to UV B light was checked for the number of Sunburn Cells under a microscope and the TNF-a levels were measured using the ELISA method. The data was then processed and analyzed using the SPSS for Windows program.

RESULTS

A total of 30 samples divided into 5 groups were studied in this research. The descriptive analysis includes mean (mean), standard deviation (SD), minimum, and maximum for the TNF-α and Sunburn Cell variables. The analysis results are presented in Table 1.

TABLE 1: Descriptive Analysis of TNF-α Variables and Number of Sunburn Cells.

Variables	Group	n	Mean	SD	Min	Max
TNF-α	Normal (P0)	6	97,88	1,19	96,79	99,69
	Placebo (P1)	6	162,41	1,87	160,00	164,96
	Treatment 1 (P2)	6	138,04	1,03	136,07	139,08
	Treatment 2 (P3)	6	124,71	2,65	121,19	128,09
	Treatment 3 (P4)	6	107,18	1,52	105,53	109,64
Sunburn Cell	Normal (P0)	6	4,00	0,13	3,80	4,20
	Placebo (P1)	6	4,07	0,27	3,80	4,40
	Treatment 1 (P2)	6	2,60	0,25	2,40	3,00
	Treatment 2 (P3)	6	1,67	0,15	1,50	1,90
	Treatment 3 (P4)	6	1,33	0,12	1,20	1,50

Analysis of treatment effects was tested based on the mean TNF-α and Sunburn Cells between groups after the administration of 2.5%, 5%, and 10% Arabica coffee leaf ethanol extract cream. The results of the significance analysis using the One Way Anova test are presented in Tables 2 and 3 below.

TABLE 2: TNF-α Levels Between Groups After Administration of Ethanol Extract Cream from Arabica Coffee Leaves.

Variables	Group	n	Mean ng/ml	Standard Deviation	*p-value*
TNF-α	Normal (P0)	6	97,88	1,19	0,000
	Placebo (P1)	6	162,41	1,87	
	Treatment 1 (P2)	6	138,04	1,03	
	Treatment 2 (P3)	6	124,71	2,65	
	Treatment 3 (P4)	6	107,18	1,52	

TABLE 3: Sunburn Cells Between Groups After Administration of Ethanol Extract Cream from Arabica Coffee Leaves.

Variables	Groups	n	Mean (cells per field view)	Standard deviation	p-value
Sunburn Cell	Normal (P0)	6	4,00	0,13	0,000
	Placebo (P1)	6	4,07	0,27	
	Treatment 1 (P2)	6	2,60	0,25	
	Treatment 2 (P3)	6	1,67	0,15	
	Treatment 3 (P4)	6	1,33	0,12	

DISCUSSION

Exposure to UV-B radiation could significantly induce the production of TNF-α. In this study, there was an increase in TNF-α levels in the group of test animals that were exposed to UV-B in the control group (P1) compared to the normal group (P0) amounting to 64.53 ng/L (65.93%) and increased significantly ($p < 0.05$).

The administration of Arabica coffee leaf ethanol extract cream significantly reduced TNF-α levels compared to the control group without extract treatment (P1). Administration of 10% Arabica coffee leaf extract cream was most effective in reducing TNF-α levels by 34.01%. In this study, it was also found that the higher the concentration of Arabica coffee leaf extract cream given, the greater the reduction in TNF-α levels. This indicates that Arabica coffee leaf extract cream is able to protect the skin as indicated by a decrease in TNF-α levels.

The Arabica coffee plants contain phenols and flavonoids which act as antioxidants hence they are able to inhibit cell damage and oxidative stress through their free radical scavenging properties, thereby reducing ROS concentrations and activating keratinocyte growth factor (KGF) receptors and tumor necrosis factor. -α (TNF-α) is suppressed. A study conducted by Hudakova et al. (2016) showed that ROS which activates signaling pathways in keratinocytes will trigger the production of TNF-α, IL-1, PGE2, IL 6, IL-8, and metalloproteinase (MMP). Inflammatory mediators (IL-8 and TNF-α) cause neutrophils and monocytes in the vasculature to enter the skin and amplify the inflammatory response (Fuller, 2019). Excessive UV radiation also causes DNA damage which is correlated with an increase in TNF-α. [9]

Administration of Arabica coffee leaf ethanol extract cream in this study significantly inhibited the increase in sunburn cells compared to the control group without extract treatment (P1). The mechanism for proving intervention is through administering ethanol extract cream from Arabica coffee leaves by comparing all dose (concentration) groups with the control group. In this study, the lowest sunburn cells occurred at a concentration of 10%. In this study, it was also found that the higher the concentration of Arabica coffee leaf extract cream given, the greater the reduction in sunburn cells.

This indicates that Arabica coffee leaf extract cream is able to protect the skin which is characterized by a decrease in sunburn cells.

There have been no studies that specifically discuss the effects of Arabica coffee leaf ethanol extract cream on sunburn cells. However, it is estimated that the inhibitory activity of increasing sunburn cells produced by the ethanol extract of Arabica coffee leaves comes from the phytochemical compound content in it. Coffee leaves contain high levels of antioxidant compounds, including flavonoids, alkaloids, saponins, and polyphenols, and have anti-inflammatory properties. [10,11] Arabica coffee leaves are known to contain the compounds chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, routine, quercetin, kaempferol, and isoquercitrin.[12]

CONCLUSION

Administration of ethanol extract cream from Arabica coffee leaves (*Coffea arabica)* can reduce TNF-α levels and the number of sunburn cells in the skin of male Wistar rats (Rattus norvegicus) exposed to UVB radiation.

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