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Short Communication

Safety evaluation and effects of cascara pulp Gayo Arabica coffee cream as anti-photoaging in animal model

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Abstract

Prolonged sunlight and ultraviolet (UV) exposure causes premature skin aging called photoaging and coffee-derived topical antioxidants may help to reduce this process. Since a significant amount of antioxidant-rich cascara pulp are wasted in coffee processing, this study aimed to evaluate the safety of cascara extract cream of Gayo Arabica coffee pulp (CECGACP) as anti-photoaging in male Wistar rats (*Rattus norvegicus*) by assessing the skin primary irritation index (PII), skin macroscopic changes and the level of tumor necrosis factor-alpha (TNF-α). Twenty-five rats were randomly divided into five different groups: negative control (base cream only), positive control containing L-ascorbic acid 3%, and three treatment groups treated with cream containing 5%, 7.5% and 10% of CECGACP. The CECGACP was extracted, formulated into cream as a topical treatment and applied on the backs of the rats two times a day, once before the UV exposure and four hours after the exposure. After four weeks, the skins were macroscopically examined, and the TNF-α levels were measured. The PII was assessed after applying the cream 24 hours before UX exposure. Our data suggested that CECGACP was considered safe because there were no erythema and edema formation on the skin of the rats with PII score of 0 (classified as no irritation or negligible). After UV exposure, all rats had visible wrinkles and erythema on the skin in particular in the first week. After administration of CECGACP, both wrinkles and erythema were decreased. The levels of TNF-α varied from 0.15±0.02 ng/mL in the negative control and CECGACP 7.5% rat groups to 0.19±0.03 in the positive control group; however, there was no significant difference among all the groups. Our study suggests that CECGACP is safe to use in Wistar rats and able to reduce the erythema and edema due to UV exposure. However, the application of CECGACP does not provide a significant reduction of TNF-α levels.

Keywords: Safety evaluation, photoaging, coffee, irritation, TNF-α

Introduction

Skin aging is a natural process of the body characterized by a decrease in the ability of the tissues to repair and maintain their normal structure and function, resulting in pathological changes in cells and tissues. This complex biological process affects all layers of the skin as well as the appearance of the individuals [1]. Skin aging is affected by internal (genetics, hormones and race) and external factors (sun exposure, pollution, lack of sleep and smoking). In particular, chronic exposure to ultraviolet (UV) causing the skin to age prematurely, called photoaging [2,3].

UV radiation that reaches the surface of the earth is mainly UVA (95–98%) and UVB (2–5%) while UVC is primarily absorbed by ozone in the stratosphere [4]. Excessive exposure to UV on

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the skin could increase the level of free radicals in the body, leading to the increase of oxidative stress, causing cellular damages and, eventually, premature aging of the skin [5,6]. Individuals who often exposed to sunlight will have a higher exposure to UV rays, making them more prone to photoaging [2] especially those in tropical countries such as Indonesia. Patients with photoaging has statistically higher risk of skin cancer development [7]. The risk of photoaging, sunburn, pigmentation and skin cancer initiation can be reduced by avoiding direct sun exposure, using sun protection [8] and using of topical antioxidants [9]. Antioxidant is a substance steady enough to grant an electron to a rampant free radical to neutralize it and antioxidants from natural ingredients are less toxic than synthetic ones [10,11].

Indonesia is known as a coffee exporter [12], produced more than 650,000 tons of coffee in 2019 [13]. Aceh, an Indonesian province, produced 41,847 tons of coffee annually [13]. Only the seed of coffee are used in the making of coffee drinks while the outer layers of the coffee fruits (husk or cascara in Spanish) become waste. The cascara however still has small amounts of variety of chemical compounds [14] including antioxidants that act as free radical inhibitors to prevent cancer and increase the physical vitality [15]. Nevertheless, research on this particular product is still limited in the health sector, and the application of cascara extract cream of Gayo Arabica coffee pulp (CECGACP) as an anti-photoaging has not been studied yet. The aim of this study was to determine the safety and the effect of CECGACP as anti-photoaging in male Wistar rats (*Rattus norvegicus*) by assessing the primary irritation index (PII), macroscopic changes, and tumor necrosis factor-alpha (TNF-α) level.

Methods

Study design and setting

An in-vivo study using animal model was conducted from February to April 2022 in the Physiology Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. Twenty-five rats were randomly divided into five groups: negative control (NC), positive control (PC), treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3). All groups were exposed with UVB and treated with different formulations of cream. NC group treated with base cream only; PC with base cream containing L-ascorbic acid 3%; T1, T2 and T3 groups with base cream containing CECGACP 5%, 7.5% and 10%, respectively. The diagram of the study groups and the treatments is presented in **Figure 1**.

Animal model and the preparation

A total of 25 *Rattus norvegicus* L. male rats were used in this study. The inclusion criteria of the rats were male Wistar strain rats, 2–3 months old, body weight ranges of 150–200 grams, healthy and active. The use of male rats was to avoid the hormonal cycle and pregnancy that could interfere the study. Before the intervention, all rats were acclimatized for one week in 50 cm x 50 cm cages and exposed to 12-hour light and 12-hour dark cycles under standard conditions (temperature of 28±2°C and adequate humidity). The rats were fed daily with the same number of pellets (Charoen Pokphand, Jakarta, Indonesia) and tap water.

Cream preparation

The coffee cascara was extracted with ethanol solvent 96% and formulated into a cream. It was made by melting the oil phase of stearic acid, cetyl alcohol, glycerin, triethanolamine, oleum rosea and liquid paraffin at 70°C. The aqueous phase was prepared separately using a methylparaben solution in heated water at 80°C. The cream base was made by adding the oil phase to the water phase, then CECGACP was added gradually while stirring until it was homogeneous. The cream compositions were made in three variations of CECGACP concentrations, which were at 5%, 7.5% and 10%. It was made by melting the oil phase of stearic acid, cetyl alcohol, glycerin, triethanolamine, oleum rosea, and liquid paraffin using a temperature of 70°C. In a separate place, the aqueous phase was prepared using a methylparaben solution in heated water at 80°C. The cream base was made by adding the oil phase to the water phase, then it was stirred until homogeneous with a homogenizer. Furthermore, while stirring, add CECGACP little by little until it was homogeneous.

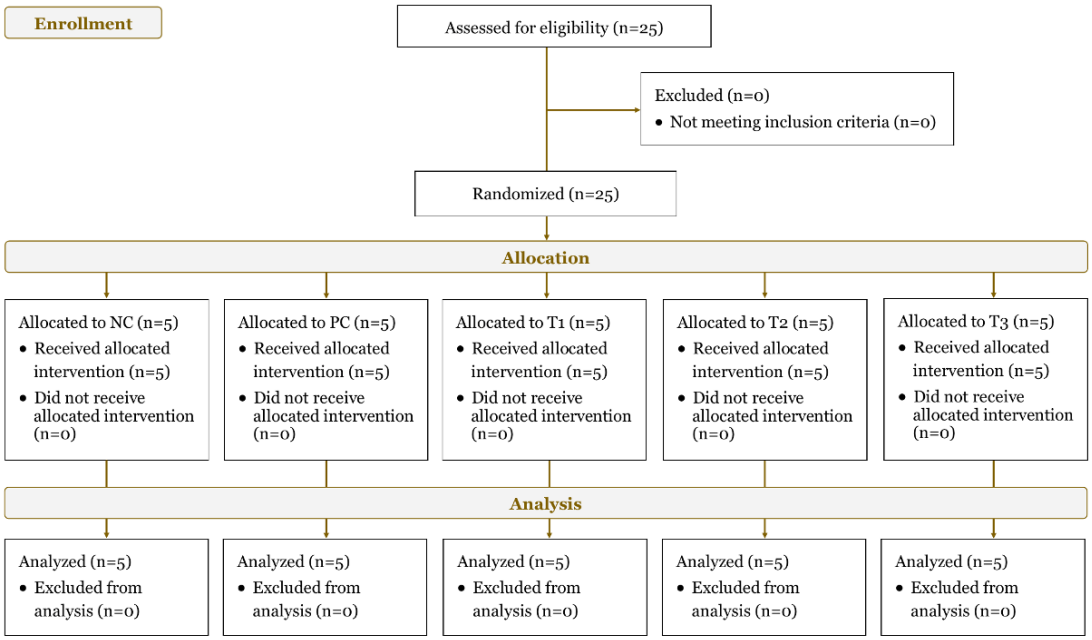


Figure 1. Diagram of the study groups and the treatments.

Ultraviolet exposure and cream treatment

The backs of all the rats were shaved with an area of 4 cm x 4 cm and were exposed to UVB from Narrowband UVB Lamp Kn-4003 (Kernel Medical Equipment, Xuzhou, China) for four weeks, with a dose of 50 mJ/cm^2 (23 seconds) in the first, 70 mJ/cm^2 (32 seconds) in the second and 80 mJ/cm^2 (36 seconds) in the third and fourth weeks. Irradiation of UVB was carried out three times a week to all the rats. The cream was applied on the backs of the rats two times a day, once just before the irradiation was conducted in the morning and then four hours after the irradiation. The cream was also applied at the same time every day on the days without the irradiation. It was then cleaned from the skin of the rats after 24 hours of the application.

Endpoints

There were three endpoints measured in this study: PPI, macroscopic observation, and the level of TNF-α. Cream safety evaluation was conducted with patch test and scored based on PII before the UVB exposure. Cream with different contents and concentrations were applied topically on shaved skin of different rats. After 24 hours, the creams were cleaned from the skin and the assessment was conducted to find whether there was any primary irritation such as erythema and or edema according to PII (**Table 1**). The scores were calculated by dividing the sum of all the erythema and edema cases at the time of observation with the number of rats multiplied with the total of the observation times to be categorized based on PII classification: no irritation or negligible (score 0.0–0.4), slight irritation (0.5–1.9), moderate irritation (2.0–4.9) and severe irritation (5.0–8.0).

Table 1. Primary irritation index (PII)

Component	Score
Erythema formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (scar formation)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema with raised margin	2
Moderate edema with raised margin (approximately 1 mm)	3
Severe edema with raised margin (more than 1 mm)	4

Macroscopic observation was conducted to examine the condition changes of the skin (wrinkles and erythema), at three different times (before, four hours after irradiation and after cream treatment). The examination of the TNF-α concentration was carried out by the skin biopsies from the back area with a size of 3x3 cm and using rat TNF-α ELISA kit (Bioenzy, Bogor, Indonesia) following the detailed procedure of the kit.

Statistical analysis

The data distribution was assessed using the Shapiro-Wilk test. Since the data were distributed normally, one-way analysis of variance (ANOVA) test was used. A $p<0.05$ was considered statistically significant. Data were analyzed using SPSS 18 (SPSS Inc., Chicago, USA).

Results

Primary irritation test and macroscopic examination

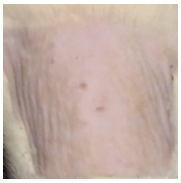
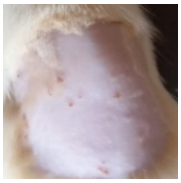
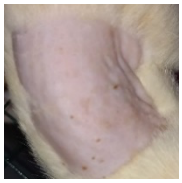
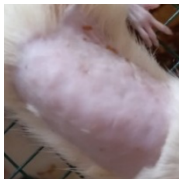
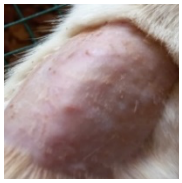
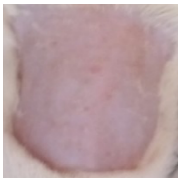
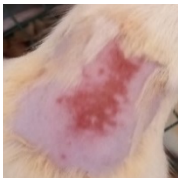
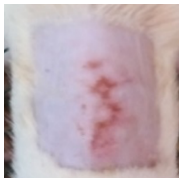
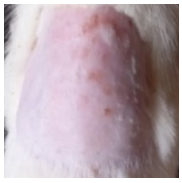
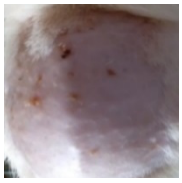
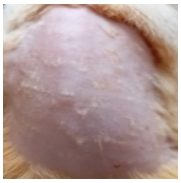
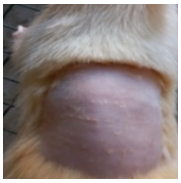
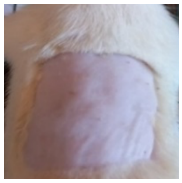
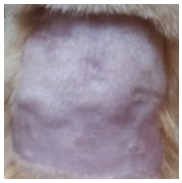
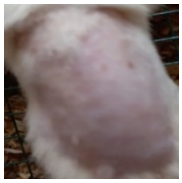
There were no primary irritations in the forms of erythema and edema found on the rats' skin after CECGACP application (**Table 2**). As there were no erythema and edema formed (score of 0.0) in all groups, therefore, the cream formulations were classified as no irritation (negligible).

Table 2. Primary irritation test results using primary irritation index (n=25)

Group	Score of formation		PII score	PII classification
	Erythema	Edema		
Negative control	0	0	0	No irritation (negligible)
Positive control	0	0	0	No irritation (negligible)
CECGACP 5%	0	0	0	No irritation (negligible)
CECGACP 7.5%	0	0	0	No irritation (negligible)
CECGACP 10%	0	0	0	No irritation (negligible)

The macroscopic examination showed wrinkles and erythema on the skin, particularly in the first week after exposure to UVB light. Nonetheless, there was a difference in the level of erythema, and the PC group experienced more extensive erythema than the other groups. After administering CECGACP, the appearances of wrinkles and erythema were decreased (**Table 3**).

Table 3. Qualitative results of macroscopic examination

Observation time	Group				
	Negative control	Positive control	CECGACP 5%	CECGACP 7.5%	CECGACP 10%
Before irradiation					
Irradiated					
After treatment					

TNF-α level

Analysis on the TNF-α concentration of the rats resulted in similar values of all groups (**Table 4**). The highest TNF-α level was found in the positive control at 0.19±0.03 ng/mL, followed by CECGACP 5% and CECGACP 10% groups at 0.17±0.03 ng/mL and 0.17±0.02 ng/mL, respectively. The rats in both negative control and CECGACP 7.5% groups had the lowest TNF-α concentration. The statistical analysis results indicated that the concentration of TNF-α were not significant different among groups (p=0.309).

Table 4. The concentration of TNF-α in rats after post-treatment (n=25)

Group	TNF-α (ng/mL) Mean±SD	F	p-value
Negative control	0.15±0.02	1.28	0.309
Positive control	0.19±0.03		
CECGACP 5%	0.17±0.03		
CECGACP 7.5%	0.15±0.02		
CECGACP 10%	0.17±0.02		

Discussion

The rats' skin that applied with formulation of CECGACP showed no irritation (no erythema and edema formation), which indicated the safety of the cream. The base cream formulation in this study was in accordance with the Indonesia National Standard with pH range of 6.7 to 7.9, and the entire ingredients of the cream used were also in accordance to the cream standards. Cream ingredients, such as high concentrations of methylparaben and triethanolamine could harm the skin by damaging the lipid membrane which inducing several mediators (e.g., phospholipase and prostaglandins) to activate vasodilation and increase the permeability of blood vessels, which in turn caused edema and erythema [16,17]. However, supplementing natural ingredients in the cream could affect more positively. Similar to the result of this study, a previous study reported that there was no skin irritating reaction from a peel-off gel mask formulated with the Lampung Robusta coffee bean extract because the pH of the gel was within skin physiological pH (pH 4.5–6.5) [18]. Another study using moringa seed extract cream also found no erythema, edema and irritation on the skin of the rats with PII at 0 [19].

There were macroscopic changes on the skin of the Wistar rats after the UVB irradiation and after the application of CECGACP. Before the exposure of UVB rays, skin of the Wistar rats was neither erythematous nor wrinkled. Directly after the UVB irradiation, erythematous skin and wrinkles were developed on the skin in all groups. After four weeks of CECGACP application, all the erythema and wrinkles were disappeared. Skin in the treatment groups appeared smoother than in the control groups. This finding is in line with previous study reported that simultaneous exposure of UVA and UVB for six weeks caused wrinkles and 12 weeks of exposure caused emerging ulcers and blisters, but no wrinkles were found on the skin treated with ginsenoside Rk3 as it contains antioxidant compounds that could inhibit the photoaging process and skin inflammation [20].

In this study, there were changes in the form of wrinkles and erythema on the skin of the rats, especially in the negative and positive control groups, compared to the skin of the rats that received CECGACP. The reason was that CECGACP contained various antioxidant compounds, such as polyphenols, flavonoids, alkaloids, tannins and saponins, which can inhibit the formation of reactive oxygen species (ROS) formation due to photoaging [21]. ROS impacts the skin by causing detriment effects on DNA through a series of stress events and it is considered as a significant contributor in the pathophysiology of photoaging [21].

The TNF-α level was not significantly different between groups, although all treatment groups had lower compared to the positive control. It was might due to the differential responses between each experimental animal and anti-inflammatory of CECGACP, such as flavonoids [22], that may not affect TNF-α, but on other pro-inflammatory cytokines [23,24].

Administration of CECGAC cream was expected to inhibit the inflammatory process associated with TNF-α induces. TNF-α is a pro-inflammatory cytokine that plays important roles in the development of various diseases by inducing apoptosis and necrosis through the activation of IkappaB kinase complex/nuclear factor-KappaB (IKK/NF-κB) and mitogen-activated protein

kinase/activator protein-1 (MAPK/AP-1) [25]. Exposure to UVB triggered ROS formation that affects the critical pathway involving TNF-α, which induces the photoaging process [22,26]. ROS formation caused damage to mitochondrial DNA and stimulated activator protein-1 (AP-1) activation. AP-1 could cause the induction of matrix metalloproteinase (MMP) expression, especially MMP-1, that leads to collagen degradation. AP-1 would also suppress transforming growth factor beta (TGF-β) causing reduced type 1 collagen synthesis leading to apoptosis [22].

Furthermore, TNF-α controls the activity of fibroblasts, vascular endothelial cells, and keratinocytes. TNF-α synthesizes extracellular matrix proteins and MMP, which are involved in tissue healing [27]. In addition, TNF-α also plays a role in cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. When infection or injury occurs, TNF-α and other cytokines such as interleukin-1 (IL-1), IL-6, IL-8 and prostaglandin E2 (PGE2) are produced by fibroblasts and monocytes. These pro-inflammatory cytokines can trigger the release of matrix metalloproteinases, decreasing the extracellular protein matrix and causing damage to collagen tissue [28]. This study is limited to the test of TNF-α only as the proinflammatory biomarker. The examination with other proinflammatory biomarkers such as IL-1, IL-6 or others that play a role in photoaging might broaden our knowledge regarding the effects of CECGACP as anti-photoaging.

Conclusion

Our study suggested that CECGACP is considered safe with PII score 0 (classified as no irritation or negligible). In addition, all three concentrations of CECGACP are able to reducing the erythema and edema of the skin after exposure with UVB. However, the application of CECGACP did not provide a significant effect in reducing TNF-α levels.

Ethics approval

This study was approved by the Veterinary Ethics Committee of Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (No 130/KEPH/XII/2021). All treatments of the animals were carried out according to the applicable standards.

Competing interests

The authors declare that there is no conflict of interest.

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Underlying data

All data underlying the results are available as part of the article, and no additional source data are required.

How to cite

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