CLITORIA TERNATEA CREAM FLOWERS EXTRACT INCREASE SKIN FIBROBLAS FROM ULTRAVIOLET B-INDUCED PHOTOAGING

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Abstract

Ultraviolet B (UVB) radiation is one of extrinsic factors that accelerates the premature aging process of the skin, which is called photoaging. Photoaging is characterized by the appearance of wrinkles due to excessive exposure to UVB rays. Clitoria ternatea flowers are one of herbal plant that have been reported for their chemical content of flavonoids. Based on the literature, flavonoids are one of the natural compounds that have potential effect as a photoprotective agents because they have the ability to absorb UVB light and can be antioxidant compounds. This research aim is to evaluate cream containing Clitoria ternatea flower extract 2.5% and 5% to increase skin fibroblas on the skin of wistar rats exposed to UVB. The indicator use number of fibroblasts in the epidermis with Hematoxylin Eosin staining. Twenty-five male white rats (Rattus norvegicus) randomly separated into five groups comprising normal control (0.9% NaCl), negatif control (UVB exposure), positif control (UVB exposure + suncreen cream), and two treatment groups (UVB exposure treated with Clitoria ternatea cream flower extract 2.5% and 5%). The findings indicated that the negative control group had the lowest average number of fibroblasts compared to the other groups. The treatment group which received Clitoria ternatea cream flower extract cream 2.5% had higher average number of fibroblasts than control negatif group. Furthermore, the treatment group which receive Clitoria ternatea cream flower extract 5% had same average number of fibroblasts as the normal control group. This study can provide information by proving that Clitoria ternatea cream flower extract 2.5% and 5% can increase the number of skin fibroblasts exposed to UVB light.

Keyword: Clitoria ternatea flowers, ultaviolet B, fibroblast, photoanging

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1. INTRODUCTION

The skin is the body's primary line of defense (Sheng et al. 2022). In this case it acts as a shield to protect the body against ultraviolet B radiation (Tanaka et al. 2004). Accumulted exposure to UVB light can cause damage to the skin structure. Furthermore, can accelerates the premature aging process of the skin, called photoaging (Geoffrey, Mwangi, and Maru 2019; Sheng et al. 2022). Reactive oxygen species (ROS) can be produced by photoaging in cutaneous fibroblasts characterized by the appearance of wrinkle (Deng et al. 2018; Saritani, Wiraguna, and Maker 2021). The most important cell type in the dermis that creates extracellular matrix, dermal fibroblasts, can be harmed by elevated ROS levels (ECM). The decrease in the population of fibroblast cells causes a decrease in collagen biosynthesis in the dermis layer (Dampati and Veronica 2020). ROS this will disrupt cell regulation which cause cell damage and even cell death. In skin exposed to UVB radiation, ROS can cause DNA damage, apoptosis, and mitochondrial abnormalities (Dampati and Veronica 2020; Sheng et al. 2022). Uncontrolled skin photoaging can lead to various dermatological diseases, including chronic optic cheilitis, solar keratosis, photoelastic fibrosis and even skin cancer (Subchan et al. 2022). It may cause the elasticity of skin's elastic fibers to degrade, leading to pigmentation, dryness, deep wrinkles, and skin laxity (Sheng et al. 2022).

One method of preventing sunburn from excessive UVB radiation is by applying sunscreen. Currently, the use of sunscreen is still dominated by chemicals composition that can caused side effect such as allergies, red skin, itching, and contact dermatitis (Puspitasari, Pratimasari, and Andriani 2019; Subchan et al. 2022). Antioxidants are frequently utilized as natural sunscreen to reduce the harmful effects of UVB exposure and shield the skin from its risks. It is commonly known that natural antioxidants from plants and fruits have fewer adverse effects than synthetic materials (Saritani, Wiraguna, and Maker 2021; Subchan et al. 2022).

Clitoria ternatea flowers is one of the most popular herbal plant nowadays. This plant is easy to grow and cultivate (Marpaung 2020). Clitoria ternatea have been extensively applied in Thailand for a variety of purposes. In South-East Asia, for examples, frequently utilized as a natural colorant in numerous drinks, cuisines, and cosmetics. Ayurveda medicine uses Clitoria ternatea in the medical field for to advance health (Kosai et al. 2015). An earlier study reported on their chemical composition such as flavonoids, fenol, saponin, tanin, steroid, etc (Chakraborthy et al. 2018; Marpaung 2020). The bioactive components of phenolic and flavonoid components in Clitoria ternatea can fight free radicals by preventing oxidation, which reduces the rate of photooxidation brought on by UVB light (Bujak et al. 2022). A prior study found that applying 5% Clitoria ternatea extract cream to Wistar rats' exposed skin inhibited the rise in MMP-1 levels and the decline in collagen levels (Saritani, Wiraguna, and Maker 2021).

There has been previous research on the protective effect of Clitoria ternatea flowers against UVB radiation, but there is still little information about its effect on preventing skin aging due to exposure to UVB rays, especially in increasing the number of fibroblasts. This is study aims to evaluate the impact of Clitoria ternatea cream flower extract to increase the number of skin fibroblasts exposed to UVB light.

2. MATERIALS AND METHOD

Experimental animals

The study was carried out at Sultan Agung Islamic University's Integrated Biomedical Laboratory School of Medicine, Semarang in November -

December 2022. This study design was experimental by using Randomized Posttest Only Controlled Group Design. The animal subjects were used 25 male rats (Rattus norvegicus) with body weight ranging from 200-250 grams and 2-3 months old. This research began after obtaining ethical approval letter from Islamic Sultan Agung University's medical school is located in Central Java with number 349/IX/2022/Komisi Bioetik. The rats were acclimated during seven days and shaved on the back in 2x2 cm size before devided into five groups. They are consis of control group (0.9% NaCl), control negatif (UVB exposure), control positive (UVB exposure and Sunscreen lotion with SPF30 PA+++), Treatment 1 group (UVB exposure and Clitoria ternatea 2.5% cream extract) and Treatment 2 group (UVB exposure and Clitoria ternatea 5% cream extract).

Drug application and UVB irradiation

Irradiation of UVB using a Polish-made Philips UVB lamp with a wavelength of 311 nm (pl-s 9w/01). UVB irradiation were given three times a week for two weeks until reach total dose 420 mJ/cm2. The rats were put in a container 40 cm away from the UVB light source. Applying the cream every 20 minutes before and 4 hours after exposure to UVB rays covering on their backs.

Extraction of total flavonoid form Clitoria ternatea

The Clitoria ternatea fresh flower collected from flower agriculture in Sleman, Yogyakarta province, Indonesia. Flowers were picked in conditions already bloom, fresh and have blue color. The drying process are refers to Cahyaningsih (2019) by using a temperature of 40°C for 3 days, then acquired some dried simplicia. The dried Clitoria ternatea flower simplicia was then ground into a powder, and 500 mL of 96% ethanol was added to the powdered simplicia in a beaker glass. After that, extraction was done for 3x3 minutes using an ultrasonic equipment. Before repeating the ultrasound, stirring is done every three minutes. To differentiate between the filtrate and macerate, the filtrate was filtered using a Buchner funnel. The collected filtrate was placed in a glass bottle. There were three treatments administered. To create a concentrated extract, the collected filtrate was concentrated at 400°C in a rotary evaporator, and then dried in a 400°C oven.

Total flavonoid levels in the extract were measured by using colorimetri (Singh et al. 2021). 1,5 ml of 2% AlCl3 (aluminium chloride) and 1,5 ml of a Clitoria ternatea flower extract solution (500 ppm in 96% ethanol) were added. It was then incubated for an additional hour at 25^{o}C. Using a UV-Vis spectrophotometer set to 420 nm to determine absorbance quantity. The amount of flavonoids overall was represented as mg QE/g extract using quercetin as the standard.

Formulation of Clitoria ternatea cream

The formulation Clitoria ternatea cream flower extract with concentration 2.5% contain 2.5 grams simplicia Clitoria ternatea ethanol extract 96% in the total mixture of 100 grams of base cream. Moreover, 5% concentration makes up the composition for the Clitoria ternatea extract cream contain 5 grams Clitoria ternatea ethanol extract in the total mixture of 100 grams of base cream. The base cream used contains 3% Sepigel, 2% lanolin, 0.5 phenoxyethanol, and 2% dimethicone.

Assessment of Fibroblast

To avoid the acute effects of irradiation, skin biopsy was performed 24 hours after the last UVB exposure. The rats were terminated to have skin tissue on their backs with a size of 2x2 cm with macro knife. The tissue blocked with liquid parafin. The number of fibroblasts was calculated through a microscope with Hematoxylin-Eosin (HE) method then view under a light microscope at 400 times

magnification. The number of fibroblast cells were viewed on a monitor and counted manually.

Statistical Analysis

SPSS was used to analyze the data, and a 95% level of confidence was used. Shapiro-Wilk test for determining normality. The results of Shapiro-Wilk test on the mean fibroblast score found that distribution was normal. One Way Anova testing is then used to find any group differences. To find significant differences among the five groups, post hoc analysis was carried out.

3. RESULTS AND DISCUSSION

Measurement of average number fibroblasts was carried out in each treatment group after 30 days of administration of Clitoria ternatea extract cream. The descriptive analysis showed that the lowest mean of fibroblast in the negative control groups was 1.9 ± 0.30, followed by positif control group was 7.8 ± 3.86, Treatment 1 groups was 9.8 ± 5.02, Treatment 2 group was 10.1 ± 2.66, and then normal control was 10.5 ± 2.64.

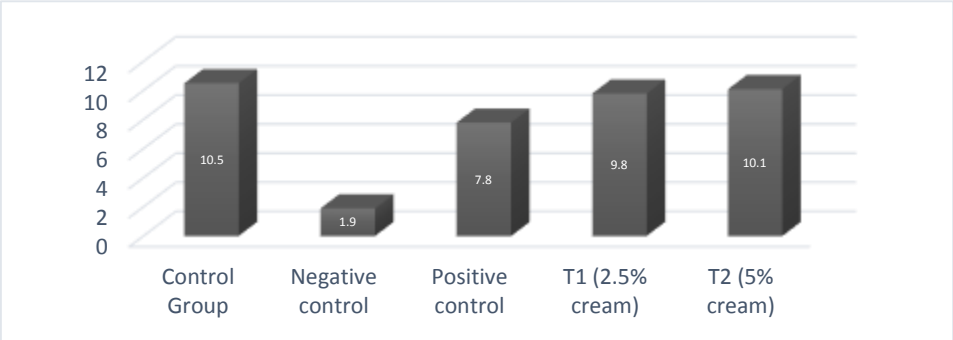

Figure 1. Result of the average number fibroblas with 5 eight-view observations form control group = 0.9% NaCl, negatif control = UVB exposure, positive control = UVB exposure and sunscreen lotion with SPF30 PA+++, T1 = UVB exposure and Clitoria ternatea cream extract 2.5% and T2 group = UVB exposure and Clitoria ternatea cream extract 5%

The results of normality test with Shapiro-Wilk test on the average score of fibroblasts found that the data distribution was normal. So that it was continued with the One-Way Anova test to obtain p = 0.00. Which indicated that there was significance in all treatment groups ($p < 0.05$). The results of the different test between groups using the Post Hoc LSD method are presented in table 1.

Table 1. Post Hoc test on parameters of the average fibroblas each group

Group		**P-value**
Control group (sham)	Negative control	0.001*
	Positive control	0.220*
	Treatment 1	0.748
	Treatment 2	1
Negative control	Positive control	0.001*
	Treatment 1	0.001*
	Treatment 2	0.001*
Positive control	Treatment 1	0.358
	Treatment 2	0.220
Treatment 1	Treatment 2	0.748

Description: * Significant with p-value <0.05

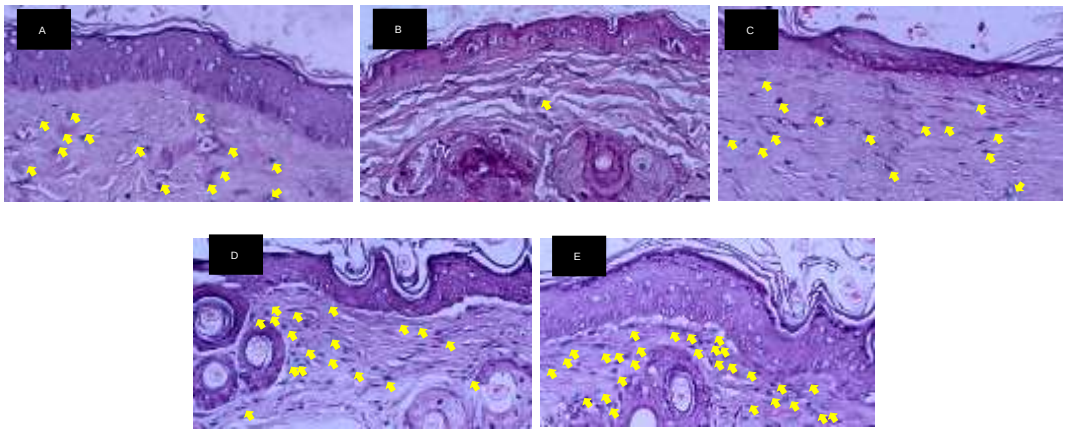

Figure 2. Microscopic appearance of Hematoxylin and Eosin staining of skin tissue under a microscope to evaluate fibroblasts. The green arrows indicate fibroblasts. Fibroblasts are distinguished from fibrocytes by their round, oval, spindle-shaped nucleus with scant cytoplasm. (A) The control group showed normal amount of fibroblast. (B) Negative control show lowest amount of fibroblast. (C) positive control group show higher amount of fibroblast than negative control group. (D, E) Treatment 1 and 2 groups show a higher amount of fibroblast than than negative control group

The differences between the normal control group, the positive control, the negative control, and treatment groups are clearly shown in Table 1. Similar findings were found in the different test between the 2.5% Clitoria ternatea cream treatment group and the negative control group ($p<0.05$). The negative control group and the treatment 2 group experienced the same findings with a value of $p = 0.001$. Meanwhile, in between the treatment 1 and 2 groups, the results were not significant, $p = 0.078$. The figure 2 describe that the negative control group that received exposure to UVB light had the least number of fibroblasts among the other groups.

According to the study's findings, there was a substantial difference between the treatment 1 group and the negative control group. It also shown in the figure 2 that amount of fibroblast in the treatment group was higher than negative control group. This indicates that the increase in fibroblasts is thought to be caused by the presence of active substances in Clitoria ternatea, specifically saponins, which can activate VEGF and increase the number of macrophages that migrate to the wound area, raising cytokine production that will activate fibroblasts in the wound tissue (Palumpun, Wiraguna, and Pangkahila 2017).

The presence of active flavonoid compounds can help wound healing due to exposure to UVB rays with a cellular mechanism, namely increasing the number of fibroblasts. Moreover, flavonoids have been shown to have anti-inflammatory properties that impact fibroblast proliferation. This substance works to decrease inflammation by preventing the production of prostaglandins from arachidonic acid and inflammatory mediators including histamine and serotonin (Ardiana et al. 2015). The tannin content in Clitoria ternatea flowers can accelerate wound healing because it has a cellular mechanism, by increasing wound closure, capillary blood vessel formation and fibroblast count (Handito et al. 2022).

The flavonoid levels in Clitoria ternatea extract cream 5% were 7421.33 mg/100g, the phenol levels were 1883.23 mg/100g GAE, the tannic acid levels were 2445.07 mg/100g TAE, the antioxidant capacity was 8719.71 Mg/L GAEAC, and the IC50 was 73.7915 ppm (Wiraguna, Dianasari, and Pangkahila 2019). This may be due to the higher levels of flavonoid compounds in the extract of Clitoria

ternatea cream which can be skin protection from UVB exposure (Wiraguna, Dianasari, and Pangkahila 2019).

Another study by Puspitasari et al., 2019 demonstrated the potential for phenolic chemicals, especially flavonoids, from the plant Clitoria ternatea used as sunscreens. Based on physical characteristics and SPF value, they investigated into the ideal Clitoria ternatea concentration for sunscreen cream. (Puspitasari, Pratimasari, and Andriani 2019).

Previous literature review research reported that burns of grade II can be treated with extracts of flavonoid components by increasing the number of fibroblasts. Active flavonoid chemicals stimulate cellular processes that accelerate up the production of fibroblasts, which contribute in wound healing. Due to its cellular mechanism, tannin content can hasten the healing of wounds by promoting wound closure, microvascular growth, and the production of fibroblasts. (Devi, Wardani, and Shantini 2021).

Previous studies conducted by Saritani et.al, 2021 found that 5% ethanol cream extract from Clitoria ternatea prevented the skin of female Wistar rats exposed to UV-B light from increasing its MMP-1 levels and decreasing its collagen content (Saritani, Wiraguna, and Maker 2021). Clitoria ternatea flowers have also been studied for their efficacy as antiinflamatory, anti-diabetic, antibacterial and antioxidant (Al-snafi 2016; Marpaung 2020). This study can provide information by proving that 2.5% and 5% Clitoria ternatea extract applied topically in the form of a cream can increase the number of skin fibroblasts. So that the benefits of Clitoria ternatea cream flower extract 2.5% and 5% are obtained as sunscreens actually protect the skin from UVB radiation damage.

The weakness of this study is that did not measure expression of proinflammatory cytokines such as TGF-β. Disruption of the TGF- pathway results in a decrease in procollagen synthesis which prevents fibroblasts from interacting with the extracellular matrix and resulted shrinks the size of fibroblast.

4. CONCLUSION

This study shows that Clitoria ternatea cream flower extract with concentration 2.5% and 5% can increase the number of fibroblas, especially to concentration 5%. Clitoria ternatea cream flower extract cream can be an alternative for suncreen from herbal plant.

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