JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v14i1.47269
JKD (DMJ), Volume 14, Number 1, January 2025 : 44-49

POTENTIAL OF BUTTERFLY PEA CREAM (*CLITORIA TERNATEA*) IN SKIN REGENERATION BY INCREASING THE NUMBER OF FIBROBLASTS AND COLLAGEN THICKNESS

Rizki Noval Nursyafillah[1], Buwono Puruhito[2], Liza Afriliana[2], Hermawan Istiadi[3], Galih Sari Damayanti[2*]
[1]Undergraduate Program, Department of Medicine, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2]Department of Dermatology, Venereology, and Aesthetics, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[3]Department of Anatomical Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

Keywords:
Clitoria ternatea,
Collagen,
Fibroblas,;
Flavonoid,
Photoagin,;
UVB.

Received: 26 September 2024
Revised: 18 November 2024
Accepted: 20 November 2024
Available online: 12 December 2024

Corresponding Author:
E-mail: drgalihsari@lecturer.undip.ac.id

ABSTRACT

Background: Indonesia is a tropical country that has a high exposure to UV radiation that causes. To overcome these effects, antioxidants are needed that can inhibit the effects of UV radiation. Clitoria ternatea, which is rich in flavonoids, is able to fight free radicals and reduce the effects of photoaging. **Objective:** This study aims to determine the effect of Clitoria ternatea extract cream on the number of fibroblasts and collagen thickness in UVB-induced male white rats (*Rattus norvegicus*). **Methods:** This study was a true experimental type with a post-test only control group design with 36 male White rats (*Rattus norvegicus*) divided into 4 groups. Clitoria ternatea extract cream was administered in 3 levels (2.5%, 5%, 10%) and Synchro® placebo base cream was applied on the rats' back for 30 days. Irradiation was performed 3 times a week, and the cream was applied twice a day. Statistical analysis was performed to see the mean differences between groups in UVB-induced male white rats (*Rattus norvegicus*). **Results:** The application of Clitoria ternatea extract cream with levels of 2.5%, 5%, and 10% can increase the number of fibroblasts significantly ($p<0.05$) from 7.40 ± 0.81 to 9.40 ± 1.39; 11.33 ± 1.73; 12.00 ± 1.49. At 5% and 10% levels can increase collagen thickness significantly ($p<0.05$) from 64.44 ± 5.64 to 70.75 ± 4.44; 77.43 ± 4.27. **Conclusion:** It can be concluded that Clitoria ternatea extract cream 2.5%, 5% and 10% can increase the number of fibroblasts and Clitoria ternatea extract cream 5% and 10% can increase collagen thickness.

INTRODUCTION

Indonesia, as a tropical country, was exposed to UV radiation throughout the day with high intensity. Among UV rays, UVB has a dominant effect on the skin, with the strongest effect in causing skin damage (photodamage) and premature aging (photoaging).[1] his handle happens through the collection of receptive oxygen species (ROS) that trigger biochemical responses, coming about in skin maturing and collagen breakdown, which causes blood vessels to widen and fiery cells to enter the tissue. ROS can harm dermal fibroblasts, in this manner diminishing extracellular network generation. In expansion, ROS moreover actuate MMP-1, a major collagenase that breaks down type-1 collagen, which at that point contributes to the onset of wrinkles.[2–6]

To overcome the effects of ROS-induced wrinkles, antioxidant substances that can prevent the formation of ROS and inhibit the clinical manifestation of wrinkles are needed.[7] Such antioxidant substances can be used as anti-aging therapies in daily skin care products.[8]

Butterfly pea flower (Clitoria ternatea) is a plant commonly used as a natural dye, traditional medicine, and ornamental plant, especially in tropical countries such as Indonesia.[9] This flower contains various bioactive substances, including tannins, saponins, flavonoids, and steroids, which are known to absorb UV light and act as antioxidant.[10] Flavonoids in telang

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v14i1.47269
JKD (DMJ), Volume 14, Number 1, January 2025 : 44-49

flowers have been shown to protect fibroblasts from free radical damage and inhibit MMP-1 activity, thus preventing collagen degradation.[11,12] This study aims to determine the effect of Clitoria ternatea extract cream on the number of fibroblasts and collagen thickness in UVB-induced male white rats (*Rattus norvegicus*).

METHODS

Animal Trials Preparation

Male white rats (*Rattus norvegicus*) of the white strain were used in the experiment. They were randomly assigned to four groups: treatment group 1 (P1), which received Clitoria ternatea cream 2.5%; treatment group 2 (P2), which received Clitoria ternatea cream 5%; and treatment group 3 (P3), which received Clitoria ternatea cream 10%. The control group was designated as group K. The rat's back hair was cut to a size of 2 by 2 cm before to therapy. The lotion was administered to the rats' backs twice a day, four hours after UVB exposure and twenty minutes before. For thirty days, UVB exposure was done three times a week for thirty minutes each time. A UVB lamp was placed twenty centimeters from the rat skin's surface.

Preparation of Clitoria ternatea Extract Cream

Clitoria ternatea extract was made from flowers using a 96% ethanol maceration procedure. Fresh Clitoria ternatea flowers were dried at 40°C for 3 days to create dry simplisia. To get the most extract, the maceration procedure was repeated three times. The filtrate that was left over was collected and concentrated using an evaporator set at 40°C. The filtrate was separated using a Buchner funnel. The obtained extract was dried at the same temperature in the oven. The extract was then subjected to 420 nm UV-Vis spectrophotometry to determine its flavonoid concentration. Synchro® base cream was used to produce Clitoria ternatea cream in three concentrations: 2.5%, 5%, and 10%.

Histopathological Examination

Rats were terminated after 30 days after being sedated with ketamine (35 mg/kgBB) and xylazine (5 mg/kgBB). Next, the rats' dorsal skin was removed and preserved in a 10% buffered formalin solution. In order to prepare the skin tissue for histopathology, Hematoxylin-Eosin (HE) staining was used. A 400x magnification microscope was used to assess the thickness of collagen and the number of fibroblasts in five fields of vision. An image analysis program that computes the thickness as a percentage was used to calculate the thickness of collagen.

Statistical Analysis

The Shapiro-Wilk normality test was used to examine the data and determine the distribution of the data, with a significance level of $p \geq 0.05$ to determine normality. The Levene test was used to perform the variance homogeneity test, and homogenous variance was indicated by $p > 0.05$. The One-Way ANOVA test was used to compare group means if the data were homogeneous and normally distributed. The Post-Hoc test was then used to determine whether there were any significant differences between the groups. The Kruskal-Wallis nonparametric test was used to continue the study if the data were not normally distributed. If needed, the Mann-Whitney test was then conducted.

RESULTS

Overview

This study evaluated the impact of a cream containing Clitoria ternatea extract on the quantity of fibroblasts and the thickness of collagen in the skin of male White rats (*Rattus norvegicus*) exposed to UVB exposure. 33 rats were used for data collection, and they were split into four treatment groups: P1, P2, and P3 received Clitoria ternatea extract cream in concentrations of 2.5%, 5%, and 10% in addition to UVB exposure, while the control group (K) received only Synchro® base cream and UVB exposure. Tests of significance were run statistically to identify any differences between the groups. These are the images of histopathology that were collected.

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v14i1.47269
JKD (DMJ), Volume 14, Number 1, January 2025 : 44-49

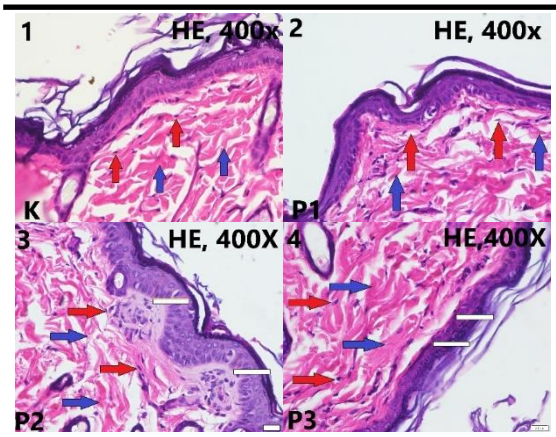

Figure 1. 1: Histopathology of K; 2: Histopathology of P1; 3: Histopatology of P2; 4: Histopathology of P3; Red Arrows : Fibroblast Cell ; Blue Arrows: Collagen Fiber

Number of Fibroblasts

The data on the number of fibroblasts in each group were found to be normally distributed (p > 0.05) using the Shapiro-Wilk test. In order to evaluate the difference between the control and treatment groups, a One-Way Anova test was used; the findings indicated that there was a significant difference (p < 0.05). The data were homogeneous, according to Levene's Test findings (p > 0.05).

The mean value and statistical test results for the total number of fibroblasts are shown in the following table:

Table 1. Descriptive Table and Normality Test for Number of Fibroblasts

Groups	Mean ± SD	p£
K	7,40 ± 0,81	0,531*
P1	9,40 ± 1,39	0,204*
P2	11,33 ± 1,73	0,169*
P3	12,00 ± 1,49	0,855*

Notes: * Normal (p>0,05); £ Shapiro-Wilk; Mean (Average); SD (Standard Deviation); p (p-value);

The data on the number of fibroblasts in each group were found to be normally distributed (p > 0.05) using the Shapiro-Wilk test. In order to evaluate the difference between the control and treatment groups, a One-Way Anova test was used; the findings indicated that there was a significant difference (p < 0.05). The data were homogeneous, according to Levene's Test findings (p > 0.05).

Table 2. Table of One-Way Anova and Levene Test Results

Groups	pμ	*Levene*
K P1 P2 P3	0,000*	0,231*

Notes: * Different average (p<0,05); * Homogenity variance (levene p>0,05); μ One-Way Anova; p (p-value);

Based on the test, it was discovered that p = 0.000 was reached by utilizing One-Way Anova, while p = 0.231 was acquired using Levene testing. It can be said that both the acquired data and the significant difference are homogeneous. Furthermore, Post-Hoc LSD testing was done to determine the differences between the groups.

Table 3. Post-Hoc LSD Results Table of Fibroblast Number

Groups		p
K	P1	0,005*
	P2	0,000*
	P3	0,000*
P1	P2	0,008*
	P3	0,001*
P2	P3	0,352

Notes: * There is a significant difference (p<0,05) Post-Hoc LSD Test; p (p-value)

Based on the test, it was found that with Post-Hoc LSD testing in group K against groups P1, P2, and P3 there were significant differences. Furthermore, in group P1 against group P2, and P3 there is also a significant difference. However, the P2 group against the P3 group did not show a significant difference.

Collagen Thickness

Table 4. Descriptive Table and Normality Test of Collagen Thickness (%)

Groups	Median (min – max)	p£
K	64,00 (56,00 – 70,00)	0,032
P1	70,00 (64,00 – 70,00)	0,000
P2	70,00 (64,00 – 80,00)	0,020
P3	80,00 (70,00 – 82,00)	0,255*

Notes: * Normal (p > 0,05); £ Shapiro-Wilk; Median (Center value); p (p-value);

The P3 group was found to be normally distributed, whereas the K, P1, and P2 groups were not (p < 0.05) according to the Shapiro-Wilk normality test. Based on these findings, a nonparametric test utilizing the Kruskal Wallis Test was used to continue the difference test.

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v14i1.47269
JKD (DMJ), Volume 14, Number 1, January 2025 : 44-49

Table 5. Kruskall Wallis Result Table of Collagen Thickness (%)

Groups	Median ± SD	p
K	64,44 ± 5,64	0,000*
P1	69,11 ± 2,03	
P2	70,75 ± 4,44	
P3	77,43 ± 4,27	

Notes: * There is a significant difference (p < 0,05); Kruskal Wallis Test; Median (Center Value); p (p-value);

Based on the nonparametric difference test using Kruskal Wallis, it was concluded that in groups K, P1, P2, and P3 there were significant differences because the p value <0,05.

Table 6. Mann-Whitney Result Table of Collagen Thickness (%)

Groups		p
K	P1	0,070
	P2	0,025*
	P3	0,001*
P1	P2	0,232
	P3	0,001*
P2	P3	0,016*

Notes: * There is a significant difference (p < 0,05); Mann-Whitney Test; p (p-value);

Based on the test, it was found that with Mann-Whitney testing in group K against group P1 there was no significant difference, while group K against groups P2 and P3 there was a significant difference. Furthermore, in group P1 against group P2 there is no significant difference. However, the P1 group against the P3 group showed a significant difference. Finally, Mann-Whitney testing was carried out on group P2 against group P3 and the results showed a significant difference.

DISCUSSION

Clitoria ternatea extract cream can significantly increase the number of fibroblasts and collagen thickness in the skin of White rats (*Rattus* norvegicus) exposed to UVB radiation. An increase in the number of fibroblasts occurred at all doses of cream administered (2.5%, 5%, and 10%), with significant differences compared to the control group. However, in terms of collagen thickness, significant increases were only observed at the 5% and 10% doses, while there was no significant difference at the 2.5% dose. Regarding the effectiveness of the creams, it was found that 5% and 10% creams were equally effective in increasing the number of fibroblasts. In addition, 2.5% and 5% creams have the same potential in increasing collagen thickness.

The bioactive ingredients in Clitoria ternatea cream, including flavonoids, phenols, saponins, tannins, and steroids, serve as powerful antioxidants that help fight free radicals and reduce the photooxidizing effects of UVB exposure. Exposure to UVB radiation is known to increase the production of Reactive Oxygen Species (ROS), which can damage fibroblasts and decrease collagen production.[13] With the flavonoids contained in the cream, the effects of free radicals can be suppressed, so that the proliferation of fibroblasts increases.[1]

In addition, Clitoria ternatea cream also plays a role in inhibiting the activity of matrix metalloproteinase (MMP), an enzyme that functions to degrade collagen. This flavonoid-induced decrease in MMP activity helps maintain collagen thickness.[14,15] These results support previous studies showing that flavonoids can reduce collagen degradation and inhibit MMP activity through inhibition of ROS-triggered signaling pathways.[16]

However, at doses of 2.5% and 5%, there was no significant difference in terms of collagen thickness, possibly due to the effect of cell saturation at certain concentrations. Creams with higher concentrations, such as at the 10% dose, may provide a stronger protective effect against collagen degradation due to the higher concentration of flavonoids. However, at a certain concentration, a cream can have a dominant function because the formulation at that number is a stable formulation. In addition, if the cream is too concentrated, it can cause an inflammatory response which can reduce the number of fibroblasts because there is a direct release of histamine cells.[17,18]

This study has limitations such as limited storage of white rat cages, so that irradiation hours cannot be carried out at one time, white rat cages are not closed so that many white rats (*Rattus* norvegicus) sometimes come out of the cage and can affect the intensity of irradiation, and the level of cream application for each rat is different and not constant because the size of the rats varies which must be improved in future studies.

CONCLUSION

It can be concluded that the administration of cream containing 2.5%, 5%, or 10% extract of Clitoria ternatea can enhance the number of fibroblasts in

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v14i1.47269
JKD (DMJ), Volume 14, Number 1, January 2025 : 44-49

White rats (*Rattus norvegicus*) that have been exposed to UVB radiation. Furthermore, the application of cream at 5% and 10% concentrations demonstrated efficaciousness in markedly enhancing collagen thickness.

ETHICAL APPROVAL

This research was approved from the Ethics Committee in Health and Medical Research (KEPK) Faculty of Medicine, Diponegoro University, Semarang, and under number 032/EC-H/KEPK-FK-UNDIP/IV/2024, the research was carried out.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

No specific funding was provided for this article, financial resources are solely independent.

AUTHOR CONTRIBUTIONS

Conceptualization, RNN, GSD; methodology, RNN, GSD, BP; data analysis, RNN; data collection, RNN, HI; source of funds, RNN, GSD; review and edit, RNN, GSD, BP, LA; supervision, RNN, GSD, BP, LA.

ACKNOWLEGDMENTS

The Department of Dermatology, Venereology, and Aesthetics at Universitas Diponegoro's Faculty of Medicine provided assistance for this study.

REFERENCES

1. Gromkowska-Kępka KJ, Puścion-Jakubik A, Markiewicz-Żukowska R, Socha K. The impact of ultraviolet radiation on skin photoaging — review of in vitro studies. J Cosmet Dermatol [Internet]. 2021 Nov 1 [cited 2024 Jan 1];20(11):3427. Available from: /pmc/articles/PMC8597149/
2. Neale RE, Lucas RM, Byrne SN, Hollestein L, Rhodes LE, Yazar S, et al. The effects of exposure to solar radiation on human health. Photochemical & Photobiological Sciences [Internet]. 2023 May 1 [cited 2024 Feb 27];22(5):1. Available from: /pmc/articles/PMC9976694/
3. Gabros S, Nessel TA, Zito PM. Sunscreens and Photoprotection. StatPearls [Internet]. 2023 Jul 17 [cited 2024 Feb 27]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK537164/
4. Lergenmuller S, Ghiasvand R, Robsahm TE, Green AC, Lund E, Rueegg CS, et al. Sunscreens With High Versus Low Sun Protection Factor and Cutaneous Squamous Cell Carcinoma Risk: A Population-Based Cohort Study. Am J Epidemiol [Internet]. 2022 Jan 1 [cited 2024 Feb 27];191(1):75. Available from: /pmc/articles/PMC8751784/
5. Kandhwal M, Behl T, Singh S, Sharma N, Arora S, Bhatia S, et al. Role of matrix metalloproteinase in wound healing. Am J Transl Res [Internet]. 2022 Jul 1 [cited 2024 Feb 27];14(7):4391. Available from: /pmc/articles/PMC9360851/
6. Kim DJ, Iwasaki A, Chien AL, Kang S. UVB-mediated DNA damage induces matrix metalloproteinases to promote photoaging in an AhR- and SP1-dependent manner. JCI Insight [Internet]. 2022 May 5 [cited 2024 Feb 27];7(9). Available from: /pmc/articles/PMC9090247/
7. Son DH, Park WJ, Lee YJ. Recent Advances in Anti-Aging Medicine. Korean J Fam Med [Internet]. 2019 Sep 1 [cited 2024 Jan 11];40(5):289. Available from: /pmc/articles/PMC6768834/
8. Maul JT, Maul LV, Kägi M, Cheng P, Anzengruber F, von Laue M, et al. Skin Recovery After Discontinuation of Long-Term Moisturizer Application: A Split-Face Comparison Pilot Study. Dermatol Ther (Heidelb) [Internet]. 2020 Dec 1 [cited 2024 Feb 28];10(6):1371. Available from: /pmc/articles/PMC7649173/
9. Oguis GK, Gilding EK, Jackson MA, Craik DJ. Butterfly Pea (Clitoria ternatea), a Cyclotide-Bearing Plant With Applications in Agriculture and Medicine. Front Plant Sci [Internet]. 2019 May 31 [cited 2024 Feb 28];10. Available from: /pmc/articles/PMC6546959/
10. Syehrin Nabila F, Radhityaningtyas D, Chandra Yurisna V, Listyaningrum F, Aini N, Pangan PT, et al. Potensi Bunga Telang (Clitoria ternatea L.) sebagai Antibakteri pada Produk Pangan. JITIPARI (Jurnal Ilmiah Teknologi dan Industri Pangan UNISRI) [Internet]. 2022 Mar 19 [cited 2024 Feb 28];7(1):68–77. Available from:

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v14i1.47269
JKD (DMJ), Volume 14, Number 1, January 2025 : 44-49

https://ejurnal.unisri.ac.id/index.php/jtpr/article/view/5738
11. Maharani, Sutrisno. Phaleria macrocarpa Flavonoid as a Potent MMP-1 Inhibitor for Endometriosis Therapy: In silico Study. Asian Journal of Health Research. 2022 Jul 27;1(2):7–11.
12. Purnamasari R, Indrastiti R, Tin Hartini M, Widiyanto B. Section A-Research paper Clitoria Ternatea Cream Flowers Extract Increase Skin Fibroblas from Ultraviolet B-Induced Photoaging Eur. Chem Bull. 2023;2023(6):2654–61.
13. Marpaung AM. Tinjauan manfaat bunga telang (clitoria ternatea l.) bagi kesehatan manusia. Journal of Functional Food and Nutraceutical [Internet]. 2020 Feb 29 [cited 2024 Aug 21];1(2):63–85. Available from: https://journal.sgu.ac.id/jffn/index.php/jffn/article/view/30
14. Guan H, Chen X, Li P, Zhang G, Kang L, Qin B, et al. Comprehensive profiling of proteome and ubiquitome changes in human lens epithelial cell line after ultraviolet-B irradiation. ACS Omega [Internet]. 2020 Dec 22 [cited 2024 Feb 28];5(50):32171–82. Available from: /pmc/articles/PMC7758888/
15. Parker JB, Valencia C, Akras D, DiIorio SE, Griffin MF, Longaker MT, et al. Understanding Fibroblast Heterogeneity in Form and Function. Biomedicines [Internet]. 2023 Aug 1 [cited 2024 Feb 28];11(8). Available from: /pmc/articles/PMC10452440/
16. Peningkatan Ekspresi Gen MMP- M, Kulit Tikus Wistar yang Terpapar Sinar Ultraviolet Prasetyowati Subchan pada B, Sofyanti Putri R, Itsnal Muna N, Magdalena Hutapea C, Cahyani E, et al. Ekstrak Bunga Telang (Clitoria ternatea L.) Menghambat Peningkatan Ekspresi Gen MMP-1 pada Kulit Tikus Wistar yang Terpapar Sinar Ultraviolet B. JMHSA: Journal of Midwifery and Health Science of Sultan Agung [Internet]. 2022 Aug 26 [cited 2024 Aug 21];1(2):13–21. Available from: https://jmhsa.id/index.php/jmhsa/article/view/22
17. Hertian R, Sani F, Farmasi J, Kedokteran F, Kesehatan I, Jambi U, et al. Uji Efektivitas Ekstrak Daun Ekor Naga (Rhaphidohora Pinnata (L.F) Schott) Terhadap Penyembuhan Luka Sayatan Pada Mencit Putih Jantan. Indonesian Journal of Pharma Science [Internet]. 2021 [cited 2024 Sep 26];3(1):11–20. Available from: https://online-journal.unja.ac.id/IJPS/article/view/13593
18. Tari M, Indriani O, Studi PS, Sekolah Tinggi Ilmu Kesehatan F, Palembang A. Formulasi Dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Sembung Rambat (Mikania micrantha Kunth). Babul Ilmi Jurnal Ilmiah Multi Science Kesehatan [Internet]. 2023 Aug 1 [cited 2024 Sep 26];15(1):126. Available from: https://jurnal.stikes-aisyiyah-palembang.ac.id/index.php/Kep/article/view/1074