

Original article**Irritation Test and Melanin Whitening Cream *Epigallocatechin Gallate* Green Tea Leaf with Mexameter and Self-Assessment**Naniek Widyaningrum¹, Fildza Huwaina Fathnin², Yuyun Darma Ayu N³, Bella N. Sari⁴**Abstract:**

Background: Green tea (*Camelia sinensis L.*) contains flavonoids consisting of *Epigallocatechin gallate* (EGCG) which functions as whitening, anti-acne, antioxidant, anti-inflammatory. Previous studies have shown that EGCG with a concentration of 6% is the optimum concentration in cream formulations (Widyaningrum, 2015b). Phase One Clinical Test was conducted to determine the safety of cream preparations with irritation as a benchmark and continued to test its effectiveness as a whitening cream on 20 respondents aged 17-45 years. **Material and Methods:** The research method employed was the Open Test. The results of the study for 21 days after applying the EGCG anti-acne cream were assessed using a mexa-meter and self-assessment. **Result:** The erythema assessment showed that the preparation did not cause irritation in the absence of erythema and for the melanin assessment, the results were not significant with p Value 0.376 (<0,05) with no changes in the melanin index. The results of the irritation test were supported by the findings of self-assessment which revealed that all self-assessment parameters were not significant and there was no irritation (p 0,317). **Conclusion:** Based on the results of the study, it could be concluded that EGCG whitening cream did not cause irritation for 21 days. The results of the melanin test formula for this whitening cream preparation were able to provide a skin whitening effect.

Keywords: *epigallocatechin gallate*; cream; skin whitening; irritation

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Introduction:

Green tea (*Camelia sinensis L.*) is a plant that is widely used as a beverage. Green tea plants contain flavonoids, polyphenols and saponins. The flavonoid content of green tea (*Camelia sinensis L.*) 80-90% contains catechins, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)¹

Epigallocatechin Gallate contained in green tea has various properties including antioxidant activity,

anti-inflammatory, antibacterial, anti-acne, skin lightening and other benefits. Previous research has proven that Epigallocatechin Gallate could be used as a skin lightener by inhibiting melagonesis and suppressing melanosome maturation activity^{2,3}. The mechanism of Epigallocatechin Gallate as an anti-acne was by inhibiting the 5 alpha-reductase type 1 enzyme in the subbasea gland so that it could inhibit excessive sebum production which could trigger acne growth⁴. Epigallocatechin Gallate was often used as

1. Naniek Widyaningrum,
2. Fildza Huwaina Fathnin,
3. Yuyun Darma Ayu N,
4. Bella N. Sari

Pharmacy Study Program, Medical Faculty, Sultan Agung Islamic University, Semarang

Correspondence: Naniek Widyaningrum *Pharmacist Profession Study Program, Sultan Agung Islamic University, Kaligawe street KM 4 Semarang 50012, Central Java, IndonesiaEmail: naniek@unissula.ac.id

the active substance of cream preparations. Topical cream preparations had the risk of irritation, edema and hypersensitivity. It was important to evaluate the irritating properties of topical preparations in humans for safety that the manufactured preparations had a good pharmacological effect and did not cause skin irritation when used by humans⁵. The first evaluation for clinical test in humans was a phase 1 clinical test^{6,7}

Previous studies have shown that EGCG is not heat stable and degrades up to 85% after exposure to UV light for 1 hour. Research conducted by Widyaningrum et al (2015)⁸ proved that the stability of EGCG would increase after the extreme method at 2°C with the addition of a pH 4 buffer solution⁹. According to Friedman et al (2009)¹⁰ research that Epigallocatechin Gallate in 8 green tea products circulating in The United States of America, Japan, and Korea showed a decrease in concentration after being stored in a dry temperature of 20°C for 6 months due to oxidation¹¹. On the other hand, Epigallocatechin Gallate was unstable to light so that Epigallocatechin Gallate would be degraded up to 85% after being exposed to radiation for 1 hour. Epigallocatechin Gallate was also stable when heated.

Phase 1 clinical tests were pre-market tests with the aim of ensuring safety during use, while one of the tests used was an irritation test. Irritation is a reaction on the skin to chemicals such as strong alkalis, strong acids, solvents and detergents. Tests in this phase were carried out on respondents with healthy skin as many as 20 respondents aged 17-45 year^{12,13}. Cream testing was conducted to determine the safety of using Epigallocatechin Gallate whitening cream on human skin. Irritation test and melanin test were carried out using a mexameter. According to research by Abdlaty and Fang (2018), the mexameter had an accuracy of about 5%, and was more sophisticated than the Dermaspectrometer^{14,15}. In addition to clinical tests using mexameter, other tests were carried out using self-assessment. According to research by Abdlaty and Fang (2018)¹⁶, self-assessment was able to describe the changes that occurred during the use of Epigallocatechin Gallate whitening cream. Self-assessment could begin with a visual assessment (VA) based on a determined ordinal degree scale. The ordinal scale of erythema varied according to the minimum and maximum redness¹⁶.

Research by Widyaningrum et al (2015)¹⁷ obtained the optimum formula of green tea cream with the

most optimal concentration of 6%. Concentration of 6% showed that the ethyl acetate fraction cream formula Epigallocatechin Gallate could inhibit *P. acnes* by affecting the dihydrofolate reductase mechanism. Furthermore, it was developed by Widyaningrum et al (2015)¹⁸ with an irritation test on albino rabbits and it was proven that the ethyl acetate fraction Epigallocatechin Gallate cream with a concentration of 6% did not cause irritation. Based on the described background, so as to determine the safety and effectiveness of using EGCG cream when applied to human skin, it is necessary to conduct a phase 1 clinical test with an irritation assessment and a melanin test for the effectiveness of the cream using a mexameter and self-assessment with the Epigallocatechin Gallate cream formula with a concentration optimum is 6%.

Materials and methods:

Instrument. A set of tools for making cream consists of a homogenizer (Ultra Turrax®), a water bath (memmert®), a porcelain cup (Haldenwanger®) and a tool for clinical testing, namely mexameter.

Ingredients. The ingredients used in this study were the optimal cream formula consisting of ethyl acetate fraction from green tea leaf extract from Sari Kemuning Inc. in Central Java, Propylene glycol, Span 80, Tween 80, Sorbitol, stearic acid, VCO, citric acid, ascorbic acid, Triethanolamine, Methyl paraben, propyl paraben.

Methods

Preparations for camellias sinensis L

The green tea leaves used were obtained from Sari kemuning Inc. in Karanganyar Central Java. Green tea leaves were cleaned, then dried with a simplicial dryer at 100°C for 5 minutes¹⁷

Extraction and Fractionation of Green Tea Leaves (camelia sinensis L)

Based on the research of Widyaningrum et al (2015)⁸, sixty grams of simplicia green tea leaves were extracted using the infusion method at a temperature of 90°C for 30 minutes with 1200 ml of distilled water. The extract was then filtered and carried out in extreme cold at 5°C. Then added vitamin C until pH 4 was stored at 2°C. Then the filtrate was fractionated with 1200 ml of ethyl acetate. The ethyl acetate fraction was evaporated with a rotary evaporator at a speed of 110 rpm at 40°C until a dry powder was obtained.

Epigallocatechin Gallate Cream Manufacturing

The oil phase, namely stearic acid, VCO, span 80 was heated at 60°C for two minutes or until well mixed. The aqueous phase, namely propylene glycol, tween 80, sorbitol, citric acid, triethanolamine, ascorbic acid, methyl paraben, propyl paraben and the rest of the distilled water was heated at 60°C for two minutes or until homogeneous then the ethyl acetate fraction of green tea extract was added for 1-2 minutes. The mixing of the oil phase and the water phase was carried out at a temperature of 60°C and then slowly lowered to a temperature of 25°C.

Clinical Test (irritation test and melanin test)

Clinical tests were conducted on 20 volunteers with healthy and normal skin aged 17-45 years¹². Epigallocatechin Gallate Whitening Cream was applied to the skin every day at night for 21 days and checked using a mexameter on day 1 and day 21 and a self-assessment was carried out.

Data analysis

Every skin condition that occurred after applying Epigallocatechin Gallate cream for 21 days was checked with a mexameter and the results of self-assessment were analyzed with SPSS.

Ethical clearance:

This research has passed the Ethics Commission of the Sultan Agung Islamic University with serial number 314/X/2020/Komisi Bioetik.

Results and discussion:

Testing of irritants and melanin in humans has received ethical approval from the Health Research/Commission on Bioethics from Sultan Agung Islamic under number 545/VIII/2019/Bioethics Commission. A study was conducted to determine the safety and effectiveness of Epigallocatechin Gallate cream in human test subjects. Initial research on the preparation of the Epigallocatechin Gallate cream fraction had been carried out at a concentration of 6% which had antibacterial activity^{8,19}. Concentration of 6% of Epigallocatechin Gallate was able to function as an anti-acne cream and can suppress the development of acne-causing bacteria, one of which was P. melanosome maturation^{2,20,21}

The stability of the formula and quality products are not enough to market the product. Safety tests must be carried out as a pre-market test before the product is marketed. Safety tests in phase 1 of clinical trials include one of them, namely irritation testing. Test the effectiveness of this cream is as a lightening. The test method used is the Open Test. The factors

observed in this method were the appearance of erythema and changes in melanin during the use of EGCG cream for 21 days. This test was carried out on 20 respondents in the vulnerable age group from 17-45 years, performed on the inner forearm. The results of the analysis were carried out using SPSS⁴

Table I The Results of Irritation Test with Mexameter

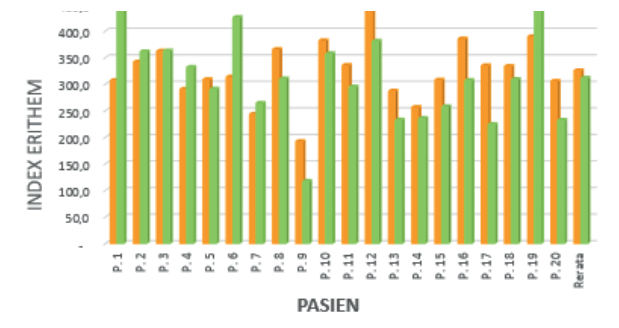


Table II p Value on Paired Sample t-test

Paired Samples Test	p Value	Description
Erythema Before – Erythema After	0.376	Not Significant

Table III The Results of Melanin Test with Mexameter

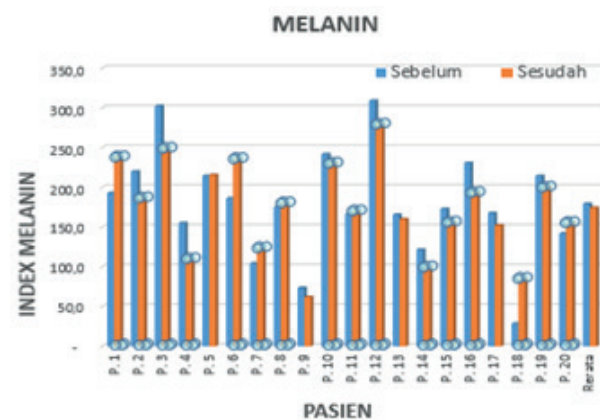


Table IV p Value on Paired Sample t-test

Paired Samples Test	Nilai p	Keterangan
Erythema Before – Erythema After	0.471	Not Significant

Table V The Results of Self-Assesment with Wilcoxon

Types of Assessments, Self-Assessment (Pre and Post Research)	P Value
Appearance	0.317
Wholeness	
Humidity	
Sensation	
Redness	
Scaly	
Fissure	
Visual	

Table I is the result of erythema on the mexameter before and after the use of Epigallocatechin Gallate cream in 20 respondents with healthy and normal skin. The use of Epigallocatechin Gallate cream for 21 days on the inner forearm did not show a hypersensitivity reaction with no marked erythema and edema. Table II SPSS test with Paired t-test, the result was 0.376, which meant that there was no significant change in erythema so that the cream did not have an irritating effect. The results of this study were in line with the research of Widyaningrum et al (2015)⁸ that in that study Epigallocatechin Gallate at a concentration of 6% ethyl acetate did not cause irritation to the skin of albino rabbits with no reddish spots on the rabbit skin, while at concentrations of 7% and 10% it could lead to toxicity. The results of this study were in line with the research of which stated that in this study green tea did not cause irritation to human skin and in the study of Sundari et al (2009)¹⁰ stated that green tea did not cause irritation and was not toxic because green tea contained many compounds. One of the polyphenols is Epigallocatechin Gallate which can be useful as an anti-irritant, a moisturizer that does not cause dry skin and other benefits. In addition, the formulation is supported by ingredients that function as moisturizing emollients so as to support the effectiveness and acceptability of the cream so that it does not cause irritation²²⁻²⁴.

Table III is the result of the melanin test using a mexameter on the same respondent and cream and SPSS analysis was carried out with the paired sample t-test listed in table IV, the result was 0.471, which meant the results were not significant. The results of the study for the melanin test showed no significant changes in melanin. The results of this study were not in line with the research of Liang et al (2014)² which stated that Epigallocatechin Gallate had a significant brightening effect because it could protect skin fibroblasts from damage caused by UVA and could reduce melanin synthesis. Research by Puspitasari et al (2017)²⁵ stated that green tea extract with a concentration of 20% in its flavonoid content had activity as a tyrosinase inhibitor so that it would prevent the formation of melanin due to UV-B rays, polyphenols in green tea could function to protect the skin from UV radiation and inhibit the process melanogenesis so that the increase in the amount of melanin was inhibited and the tannin content in green tea could function as a lightener with an anti-

tyrosinase mechanism so that melanin production could be inhibited. The results of this study lacked effectiveness as a lightening agent²⁶⁻²⁸. Limitations and shortcomings in this study were the activities of respondents who were exposed to sunlight so that it could inhibit the work of epigallocatechin gallate lightening cream and the inhibition of the malanogenesis process was less than optimal. Researchers suggested quarantine at the time of the study so that the respondent's activities could be controlled^{29,30}.

Table V is the SPSS results for self-assessment, the results were 0.317 for all parameters such as appearance, integrity, moisture, visual, gaps, scales, sensation, redness, which meant that the results of self-assessment were not significant. The results of this study proved that all parameters were not significant with no marked changes in the dosage form. This study was in line with the research of Widyaningrum et al (2015)⁸ that the optimum formulation of Epigallocatechin Gallate cream with a concentration of 6% remained stable in storage and did not cause changes in skin conditions on the skin of albino rabbits with no signs of redness, scaly and fissures or irritation^{31,32}.

Conclusion:

Epigallocatechin Gallate cream green tea leaf extract does not cause skin irritation, nor trigger erythema, Epigallocatechin gallate cream has a skin whitening effect.

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Conflict of Interest

The Authors declared there is no conflict of interests.

Authors's Contribution

Contribution of Authors Data gathering and idea owner of this study : Widyaningrum N, Fathnin FH
Study design : Widyaningrum N, Ningrum YDA

Data gathering : Sari BN Writing and submission of manuscript : Fathnin FH, Sari BN Editing and approval of final draft : Ningrum YDA, Fathnin FH

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