Study of Anti-Aging Effectiveness and Irritation of Day Cream Containing Tetrahydrocurcumin

By Agus Siswanto
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ABSTRACT

Aim: The aim of this study was to determine the anti-aging effectiveness and the irritation effect of a day cream containing tetrahydrocurcumin.

Methods: The anti-aging activity test used a pre-test post-test control group design in mice (Mus musculus L.). The shaved dorsal skin of the mice was applied with the tested creams, i.e. the cream contain tetrahydrocurcumin (THC), ethyl ascorbyl ether (positive control, PC), and the cream base (negative control, NC). At 2 hours before and 15 minutes after UV irradiation, the sensitivity, collagen level, elasticity, and water content of the skin was measured by a skin analyzer. This process was repeated daily in 8 weeks. The skin condition at the beginning and at the end of the treatment was compared. The irritation test of THC, PC, and NC used a HET-CAM method. The hemorrhage time, lysis time, and coagulation time of the hen’s egg was calculated using irritation score (IS) to get irritation index value.

Results: The group with the cream containing THC treatment showed a significant difference in sensitivity, collagen level, elasticity, and water content before and after the treatment (p ≤ 0.05). PC and THC showed no sensitivity (0 mm² of erythema). THC showed the highest collagen level (80.17±6.4%), elasticity (66±6.07%), and water content (22±2.32%).

Conclusion: Based on the scores of all the anti-aging parameters, the THC cream had the same anti-aging effectiveness as PC and more effective than NC. The cream containing THC was effective as an anti-aging and was not irritate.

Keywords: anti-aging, tetrahydrocurcumin, skin analyzer, HET-CAM

INTRODUCTION

The most visible sign of aging in the skin is the uneveness of skin color, wrinkles, and loss of skin elasticity. Although chronological aging cannot be prevented because it naturally matches age, photoaging can be prevented by using anti-aging cosmetics.

An anti-aging product usually containing antioxidants and a substance that acts directly in the regulation of cells associated with the aging process. Tetrahydrocurkumin (THC) is one of the main metabolites of curcumin. The water solubility of THC is higher than curcumin. Unlike curcumin, THC is white so have the potential to be made into cosmetic preparations.

Seeing the good THC potential to be made into an anti-aging preparation, THC will be made into a day cream preparation. To see the usefulness and safety of the THC day cream, this study tested the effectiveness of anti-aging and irritation tests.

MATERIALS AND METHODS

Materials
Day cream containing 2% THC, day cream base, ethyl ascorbyl ether (Sigma), NaCl, NaOH (Merck), mice, and hen’s eggs.

**Equipment**

UVA and UVB Repti Glo 13 W lamps from Exoterra®, analytic balance (Adam AFA-210 LC, USA), Skin Analyzer EH 900 U, incubators.

**RESEARCH METHODS**

**Anti-aging effectiveness test using EH 900 U skin analyzer**

The anti-aging study was conducted using non-invasive biophysical methods. The anti-aging parameters include skin sensitivity, moisture content, collagen level, and elasticity, measured by Skin Analyzer EH 900 U. The application of the test cream was carried out at 2 hours before UV irradiation containing UVA 630 μW/cm² and UVB 105 μW/cm². Then 15 minutes after UV irradiation, the application of the cream was repeated. The process was carried out every day for 8 weeks, then the anti-aging parameters were again measured [8]. Data results before and after treatment were analyzed statistically with SPSS 16 program.

To determine which treatment group had the greatest anti-aging effectiveness, scoring each anti-aging parameter was done by comparing the value of each anti-aging parameter between all test groups (NC, PC, and THC day cream).

The hatched hen’s eggs for 9 days and had blood vessels grouped randomly into 5 groups with 3 replications. The five groups were 0.9% NaCl, 0.1 N NaOH, NC (cream base), PC (ether ascorbyl ether cream), and THC day cream. A small portion of the egg was opened, then given cream as much as 0.3 gram/0.3 mL per test group on the chorioallantoic membrane which contained lots of blood vessels. After standing for 5 minutes, changes in blood vessels were observed. The data obtained from the irritation test were hemorrhage time, lysis time, and coagulation time were put into the irritation score (IS) equation and calculated to obtain the irritation index.

Note: Time of bleeding (hemorrhage) is time to start bleeding (seconds). Lysis time is time starts lysis (seconds). Time of denaturation is time starts coagulation (seconds).

**RESULTS AND DISCUSSION**

**Anti-aging effectiveness test using EH 900 U skin analyzer**

The results of biophysical measurements of erythema, sensitivity, water content, collagen level and elasticity of dorsal rats skin before receiving treatment and after 8 weeks of treatment are shown in Table 2.

The biophysical methods in anti-aging studies are more acceptable because of less painful, so the test subjects are more cooperative and produce more reproducible data. This method can produce qualitative and quantitative data and can be used for statistical tests.

**Skin sensitivity**

Intense exposure to solar UV rays can cause sunburn and skin to become sensitive. The prolonged exposure can damage the dermis structure and cause premature aging of the skin. UVB rays can cause inflammation of the skin by activating inflammatory mediators such as a cytokine, vasoactive, and neuroactive to produce an inflammatory response.

The number of erythema before and after treatment can be seen in Table 1. Before the treatment, the number of erythema throughout the test group was 0 (no sensitivity). After 8 weeks of treatment, NC had the number of erythema 1.33, while PC and THC day cream did not experience sensitivity. There was no significant difference (p > 0.05) in the number of erythema between each group.

The diameter of erythema (sensitivity) before and after treatment can be seen in Table 1. Before the treatment, the diameter of erythema in the entire test group was 0 mm² (no sensitivity). After 8 weeks of treatment, NC had an erythema with a diameter of 0.06 mm².
This was significantly different (p < 0.05) both with PC and THC day cream that showed no sensitivity.

The use of antioxidant substances can provide a protective effect from UV radiation such as erythema [14]. UVB will induce ROS and produce free radicals which can further promote downstream signal transduction in skin fibroblasts. This causes DNA damage directly or indirectly and induces an inflammatory response. THC protects the skin and cell viability from UVB radiation by inhibiting ROS and phosphorylated mechanisms. Thus THC can reduce skin damage and inflammation due to UVB by modulating inflammatory expression [15]. So NC which was given cream without antioxidants experienced skin sensitivity, while PC and THC day cream did not.

Based on the statistical analysis, there were not significant difference (p > 0.05) in sensitivity values after - before treatment between the three test groups (figure 1).

Water content

The decrease in water content can cause loss of skin moisture. So that the skin looks dry, this is one of the markers of the aging process [16]. PC and THC day cream moisture content increased after 8 weeks of treatment (Figure 3).

Other than increased the water content, THC gives a smoother, brighter skin appearance than PC. The color of the PC appears darker than THC. This is because THC efficiently inhibits tyrosinase so as to provide an enlightening effect on the skin [17]. THC can increase water content through increased levels of Hyaluronic acid (HA).

PC and THC experienced an increase in water content significantly (p < 0.05) after 8 weeks of treatment compared to pre-treatment. Both water content increases were significantly different compared to NC, but the THC gave the higher increase significantly compared to PC (P < 0.05).

Collagen levels

Repeated UVB exposure will activate enzymes that degrade collagen and inhibit collagen production through increased MMP-1 expression. MMP-1 is the main mediator for the emergence of collagen degradation in the skin that has photoaging. Collagenolytic MMP-1 enzyme degrades collagen elastin fibrils which are important for maintaining skin elasticity.

The collagen level of PC and THC was significantly increased after the treatment for 8 weeks (p < 0.05) compared to the pre-treatment. Otherwise, the collagen level of NC was decreased significantly. The increased collagen level after-before the treatment of the PC and THC were significantly different (p < 0.05) compared to the NC. But the PC and THC increased collagen level was not significantly different.

The percentage of collagen level was indicated by the presence of a bright blue color in the image displayed by Skin Analyzer EH 900 U software, as seen in Figure 4. The greater the collagen level, the more uniformly the bright blue color was seen. This was as seen in Figure 4, where THC and PC day cream had a bright blue color after treatment which looks more evenly distributed than NC.

Elasticity

Elastin is a major component of extracellular membranes (ECM) along with collagen which has an important role in maintaining skin elasticity. One result of loss and loss of skin elasticity is the occurrence of wrinkles on the skin which is a sign of aging [15, 19].

The elasticity is indicated by the light blue in the image (Figure 5). THC and PC experienced an increase in skin elasticity compared to NC. The percentage of skin elasticity is indicated by the presence of light blue in the analysis using Skin Analyzer EH 900 U. If the skin surface is more elastic, the more light blue is visible. This is as seen in Figure 6, where THC and KP day cream which has increased elasticity, has a light blue color after treatment which looks more evenly distributed than NC. NC has decreased elasticity after treatment.

THC can significantly increase elastin synthesis, so that skin elasticity increase. THC also has antioxidant protection that can protect the skin to remain elastic after exposure to UV radiation.

Based on statistical analysis, the elasticity of NC, PC, and THC after treatment differed significantly compared to before treatment (p < 0.05). PC and THC elasticity increased significantly after treatment for 8 weeks (p < 0.05). NC showed decreased elasticity significantly (p < 0.05).
The difference of elasticity after and before treatment between PC and THC were significantly different from NC (p < 0.05). PC was not significantly different from THC (p > 0.05).

**Scoring**

The score for the number of erythema in all test groups was 0 (zero) because the Kruskal-Wallis p-value test was 0.120 (p > 0.05), so there was no difference in the number of erythema in all variants of the test group.

Collagen level scores can be seen in Table 3.

Based on collagen level scoring in Table 3, it shows that PC had a significantly better collagen level than NC. THC had significantly better collagen levels than NC. THC collagen levels did not differ significantly compared to PC.

Based on elasticity scoring in Table 5, shows that PC had a better elasticity than NC. THC day cream had significantly better elasticity than NC. THC elasticity did not significantly different than PC.

Based on the water content scoring in Table 5, it shows that PC had a significantly better moisture content than NC. THC day cream had significantly better water content than NC. THC water day cream levels did not differ significantly compared to PC.

Based on the summation of scoring results from all anti-aging parameters (number of erythema and erythema diameter, collagen level, elasticity, and water content), it can be concluded that THC day cream had greater anti-aging effectiveness than NC and was equivalent to PC.

**Irritation test with the HET-CAM method**

The principle of the HET-CAM method was to observe existing vascular changes, such as hemorrhage, vascular lysis, and coagulation. The chorioallantoic membrane (CAM) is a complete network containing arteries, veins, and capillaries [11]. In this irritation test using 0.1 N NaOH as a control of irritant because it is a strong base that can cause irritation. NaCl 0.9% is used as a non-irritant control because it is a physiological salt that does not cause irritation.

The presence of irritation was indicated by damage to the chorioallantoic membrane, where the chorioallantoic membrane given by NaOH 0.1 N (0.1 N NaOH) was hemorrhage, lysis, and coagulation.

Based on the calculation using the irritation score (IS) equation, the value of the irritation index is shown in Table 7. NaOH 0.1 N showed an irritation index of 9.03, which was included in the irritating group. NaCl 0.9%, PC, NC, and THC has an irritation index of 0, so they did not cause irritation. It can be said that THC day cream does not cause irritation and is safe to use.

**CONCLUSION**

THC day cream had greater anti-aging effectiveness than NC and had same anti-aging effectiveness as ethyl ascorbyl ether day cream. THC day cream safe to use and does not irritate.
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**ORIGINALITY REPORT**

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