

Study on the Repairing Effect of Cosmetics Containing *Artemisia annua* on Sensitive Skin

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Abstract

In view of the increasing sensitivity of consumer skin in recent years, cosmetics containing *Artemisia annua* extract was tested to evaluate its effectiveness in repairing sensitive skin. Through the experiment of xylene-induced ear swelling in mice, it was found that the inhibition rates of ear swelling in mice induced by xylene in three groups of cosmetics containing *Artemisia annua* extract reached 60.40%, 73.36% and 74.01%, respectively, close to the positive drug group. Twenty-five sensitive skin volunteers were selected for human clinical trial, and the skin TEWL value, cuticle hydration degree and skin heme (ultra-high concentration) were tested. The results showed that using cosmetics containing *Artemisia annua* extract for four weeks could effectively increase the hydration degree of cheek cuticle by 63.90% and reduce transepidermal waterloss (TEWL) by 21.51%. The skin heme (ultra-high concentration) decreased by 69.14% and the affected area decreased by 77.47%. The results show that the cosmetics containing *Artemisia annua* extract can inhibit inflammation, repair skin barrier, improve damaged skin, and reduce redness and other sensitive skin symptoms.

Keywords

Sensitive Skin, *Artemisia annua* Extract, Transepidermal Waterloss (TEWL), Cuticular Hydration, Inflammation, Heme, Repair

1. Introduction

In recent years, the concept of sensitive skin (Sensitive Skin) has been widely recognized by consumers and personal product manufacturers, and more and more recognized by dermatologists [1]. SS is a kind of sensitive disease with high skin conditioned response, low tolerance and susceptibility to external irritants

[2]. Compared with normal skin, sensitive skin (Sensitive Skin) shows erythema (redness, swelling, inflammation), burning sensation, itching, tingling or tingling sensation, dry, tight and uncomfortable skin. Skin sensitivity is usually caused by the following factors: physical damage of the skin (including ultraviolet radiation, extreme temperature), chemical stimulation, psychological stress response, hormone drug induction, etc. [1] [2] [3]. The mechanism of sensitive skin involves the complex process of skin barrier, neurovascular and immune inflammation [4]. The impairment of skin barrier function is an important reason for skin sensitivity [5]. Due to the imbalance of intercellular lipids, the skin barrier function of sensitive skin decreased, TEWL was higher than that of non-sensitive skin, and the hydration degree of cuticle of facial skin was lower than that of normal skin [6]. Under stimulation, the TWEL of sensitive skin was significantly higher than that of non-sensitive skin [7] [8], and the hydration degree of stratum corneum decreased significantly [9]. The detection of skin transepidermal waterloss (TEWL) and cuticular hydration can be used as indexes to evaluate skin barrier function [10]. The microcirculation of sensitive and damaged skin (blood circulation in capillaries or small blood vessels) can be increased or destroyed [11], resulting in erythema (swelling, inflammation). The detection of heme (ultra-high concentration) by Antera3D imaging equipment can be used as a quantitative index of skin erythema index. Skin erythema index can quantify a certain degree of skin barrier activity [2], as an evaluation index of sensitive skin [10].

The active ingredient of *Artemisia annua* extract is artemisinin. *Artemisia annua* has a long history as an antimalarial drug. In addition to its effective antimalarial activity, artemisinin and its derivatives have also been shown to affect the immune response [12] [13]. In recent years, Wang Junxia, Huang Li, Zhang Lijun and Tan Li have also studied the effects of artemisinin on skin inflammation and immune regulation [14] [15] [16] [17]. Yu Wanying and Yang Huiliang studied the anti-inflammatory effect and mechanism of artemisinin and its derivatives [18] [19].

With the development of a large number of clinical and research work, artemisinin has been used in antimalarial drugs, but there are only a handful of applications in the field of cosmetics. In this study, a series of cosmetics with *Artemisia annua* extract were used to carry out xylene-induced ear swelling test and human clinical efficacy test, and to evaluate its anti-inflammation, repair skin barrier damage and reduce sensitive muscle redness.

2. Material

2.1. Main Reagents and Instruments

Xylene, Chengdu Cologne Chemical Reagent Factory; compound Dexamethasone Acetate Cream, Xinhecheng Holdings Group Co., Ltd.; KM Mouse, 18 - 22 g, provided by Animal Center of Sichuan Academy of traditional Chinese Medicine. CARE ARMOUR soothing repair toner, CARE ARMOUR soothing repair

essence, CARE ARMOUR soothing repair lotion, CARE ARMOUR soothing repair cream, blank paste (Yinsha Cosmetics Co., Ltd.).

Lotions and creams contain *Artemisia annua* extract with the same efficacy. Human clinical tests use cream during the day and lotion at night. VapoMeter transdermal water loss meter, MoistureMeterSC skin cuticle hydration meter, Finland Delfin company, Antera3D imaging equipment, miravex, Ireland.

2.2. Method

2.2.1. Ear Swelling Test of Mice Induced by Xylene

1) Dose grouping

Sixty mice were randomly divided into 6 groups with 10 mice in each group. The mice were divided into control group, positive group (compound dexamethasone acetate cream) and four dose groups of 1 ml sample. Sample 1 (control group), sample 2 (CARE ARMOUR soothing repair cream), sample 3 (CARE ARMOUR soothing repair moisturizer lotion), sample 4 (CARE ARMOUR soothing repair moisturizing cream) (Yinsha Cosmetics Co., Ltd.). The soothing repair moisturizer lotion, soothing repair cream, soothing repair moisturizing cream three kinds of products are all contain *Artemisia annua*, There are in order to adapt to different season and different consumer, the oil is different only on these 3 formulations.

2) Experimental step

The control group was smeared with distilled water (50 μ l/per mouse) on both sides of the right ear of mice, while the administration group and the positive group were smeared with samples (0.2 g/per mouse) on both sides of the right ear of mice according to the group, and coated continuously for 15 days.

One hour after the last administration, xylene (0.03 ml/) was evenly smeared on both sides of the right ear to cause inflammation. 30 minutes later, the animals were killed and the thickness of both ears was measured. The swelling inhibition rate (%) was calculated by the difference of the thickness of the two ears (mm); and the difference of the thickness of the two ears (mm).

Results there was a significant difference in the degree of swelling among the three groups by t-test, and the test level was $\alpha = 0.05$.

Swelling degree (mm) = right ear thickness (mm) – left ear thickness (mm)

Swelling inhibition rate (%) = (– control group ear thickness difference (mm) – sample group ear thickness difference (mm))/ blank control group ear thickness difference (mm) \times 100%.

2.2.2. Human Trial Efficacy Test

Volunteers first fill in the “trial skin questionnaire” according to the requirements, and select 25 female volunteers who reported their skin as dry skin and sensitive skin (redness, itching, edema, eczema and other allergic skin) and the age between 18 - 50 years old, fill in the “commitment to voluntarily participate in the human trial test of cosmetics” and promise to participate in the human trial period. Every day, we can use the product timely and accurately according

to the instructions, and do not use other cosmetics during the trial test (cleansing and sunscreen are not included). The order of use of the product is to use CARE ARMOUR soothing toner, CARE ARMOUR soothing repair essence, CARE ARMOUR soothing repair lotion or CARE ARMOUR soothing facial cream after cleansing. The follow-up tests were performed at 0 week, 1 week, 2 weeks and 4 weeks. Before each test, the subjects washed their faces with facial cleanser and gently dried their faces with tissue paper. After sitting in the environment of constant temperature and humidity (25 ± 2) °C and relative humidity (55 ± 10)%, the test was carried out by professionals after 30 min.

Skin transepidermal waterloss (TEWL) measurement: the right cheek transepidermal waterloss (TEWL) was measured by VapoMeter transcutaneous water loss meter, and the average value was measured for 5 times.

Measurement of skin cuticle hydration: the hydration degree of right cheek and right mouth cuticle was measured by MoistureMeterSC skin cuticle hydration meter, and the average value was measured for 5 times.

Facial imaging: Antera3D imaging equipment was used to take pictures of the subjects' faces (left and right cheeks, left and right eyelids). The pictures of heme (ultra-high concentration) model before 0 week treatment and 4 weeks (28 days) after using the product were compared and analyzed, and the data of ultra-high heme concentration and affected area were collected.

Statistical methods: SPSS20.0 statistical software was used for data analysis. The normal distribution and homogeneity of variance of the parameters were tested before statistical analysis. T test was used to compare the confidence interval of 0 week value, 1 week value, 2 week value and 4 week value, and the significance level was 95% ($P < 0.05$).

3. Results and Discussion

3.1. Effect of Cosmetic Samples on Xylene-Induced Ear Swelling in Mice

15 days after four cosmetic samples were coated on the right ear of mice, no obvious irritating effect on the skin of mice was found. From the data of the thickness of mouse ear slices, it can be seen that compared with the control group, the cosmetic sample group can reduce the effect of xylene-induced ear swelling (ear thickness), and the degree of swelling is significantly reduced. There was no significant difference in the inhibitory and anti-inflammatory effect of the sample group without *Artemisia annua* extract (blank paste). The inhibitory and anti-inflammatory effects of the samples 2, 3, 4 were statistically significant ($P < 0.01$), and the swelling inhibition rates reached 60.40%, 73.36% and 74.01% respectively. The swelling inhibition rate of positive the sample containing *Artemisia annua* extract was close to that of positive compound dexamethasone acetate cream. It can be concluded that the sample containing *Artemisia annua* extract has good anti-inflammatory effect and can inhibit the ear swelling induced by xylene in mice. and there are no possible side effects caused by positive com-

pound dexamethasone acetate cream. The experimental results are shown in **Table 1**.

3.2. Human Trial Efficacy Test

3.2.1. Volunteer Statistics

40 volunteers were recruited, of whom 25 met the criteria, all female, aged between 18 and 50 years old, with an average age of 35.4 years.

3.2.2. Measurement of TEWL Value of Skin Transepidermal Waterloss

The TEWL value of right cheek was measured by VapoMeter transepidermal waterloss meter 0 weeks before and 1 week, 2 weeks and 4 weeks after the trial of the product. The test results are shown in **Table 2**.

It can be seen from **Table 2** that after using CARE ARMOUR series cosmetics containing *Artemisia annua* extract for 1 week, 2 weeks and 4 weeks, the TEWL value showed a downward trend, and after 4 weeks of use, the TEWL value decreased by 21.51% compared with 0 weeks, with significant statistical difference ($P < 0.05$). The TEWL value test data of this human trial show that CARE ARMOUR series cosmetics containing *Artemisia annua* extract can strongly improve, repair and improve the skin barrier function, thus improving the condition of sensitive skin.

3.2.3. Skin Cuticle Hydration Test

The hydration of horny layer of right cheek and right mouth was measured by

Table 1. The effect of cosmetic samples on xylene-induced ear swelling (ear thickness) in mice.

| Groups | Size of animal | Degree of swelling (ear thickness, mm) | Swelling inhibition rate (%) |
|---|----------------|--|------------------------------|
| Control group | 10 | 0.400 ± 0.087 | - |
| Sample 1 | 10 | 0.335 ± 0.073 | 16.31 |
| Sample 2 | 10 | 0.158 ± 0.069** | 60.40 |
| Sample 3 | 10 | 0.107 ± 0.087** | 73.36 |
| Sample 4 | 10 | 0.104 ± 0.047** | 74.01 |
| Positive group (compound dexamethasone acetate cream) | 10 | 0.024 ± 0.069** | 93.91 |

Note: compared with the control group, **means $P < 0.01$.

Table 2. The transcutaneous water loss TEWL ($\text{g}/\text{m}^2/\text{h}$) data.

| Test time | TEWL value ($\text{g}/\text{m}^2/\text{h}$) | Reduction rate (%) |
|-----------|---|--------------------|
| 0 week | 25.46 ± 8.93 | - |
| 1 week | 25.19 ± 9.42 | 1.07 |
| 2 week | 21.95 ± 6.92 | 13.78 |
| 4 week | 19.98 ± 4.78* | 21.51 |

Note: compared with week 0, *means $P < 0.05$.

MoistureMeterSC skin cuticle hydration meter 0 weeks before and 1 week, 2 weeks and 4 weeks after the trial of the product. The test results are shown in **Table 3** and **Table 4**.

As can be seen from **Table 3** and **Table 4**, after using facial products containing *Artemisia annua* extract for 1 week, 2 weeks and 4 weeks, the hydration degree of the stratum corneum of the cheek and the corner of the mouth increased significantly, and the hydration degree of the cuticle of the right cheek increased by 63.90%, 79.32% and 73.81% respectively compared with 0 weeks, with a very significant statistical difference ($P < 0.01$). Compared with the 0-week value, the 1-week and 2-week values of the cuticular layer of the right mouth increased by 46.27% and 48.05% respectively, with significant statistical difference ($P < 0.05$), and the 4-week value increased by 90.32% compared with the 0-week value. There was a very significant statistical difference ($P < 0.01$). To sum up, according to the test results of the hydration degree of the stratum corneum after the trial of this product, we can know that the CARE ARMOUR series cosmetics containing *Artemisia annua* extract can greatly increase the moisture content of the stratum corneum after one week of use, and has a lasting hydrating and moisturizing effect. It can alleviate the dryness of sensitive skin, significantly improve the function of skin barrier and deeply moisturize sensitive skin.

3.2.4. Comparison of Antera3D Imaging Images

Antera3D imaging equipment takes pictures and analyzes them in heme (ultra-high concentration) mode. Ultra-high heme concentration and affected area are particularly suitable for sensitive skin, barrier damaged skin, wound healing and inflammation research. Compare and analyze the skin imaging pictures before and after use to judge whether the product has the effect of repairing sensitive skin, damaged skin and anti-inflammation. Some clinical pictures of heme (ultra-high concentration) are shown in **Figures 1-6**.

Table 3. The right cheek stratum corneum hydration (A.U) data.

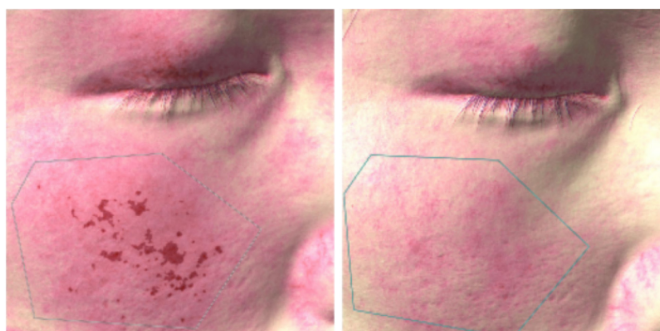
| Test time | Stratum corneum hydration (A.U) | Increase rate % |
|-----------|---------------------------------|-----------------|
| 0 week | 44.95 ± 20.88 | - |
| 1 week | 73.67 ± 24.98** | 63.90 |
| 2 week | 80.60 ± 30.31** | 79.32 |
| 4 week | 78.13 ± 26.35** | 73.81 |

Note: compared with week 0, *means $P < 0.05$, **means $P < 0.01$.

Table 4. Statistics on the hydration degree (A.U) of the stratum corneum in the right mouth.

| Test time | Stratum corneum hydration (A.U) | Increase rate % |
|-----------|---------------------------------|-----------------|
| 0 week | 37.50 ± 23.46 | - |
| 1 week | 54.85 ± 29.16* | 46.27 |
| 2 week | 55.52 ± 33.90* | 48.05 |
| 4 week | 71.37 ± 30.64** | 90.32 |

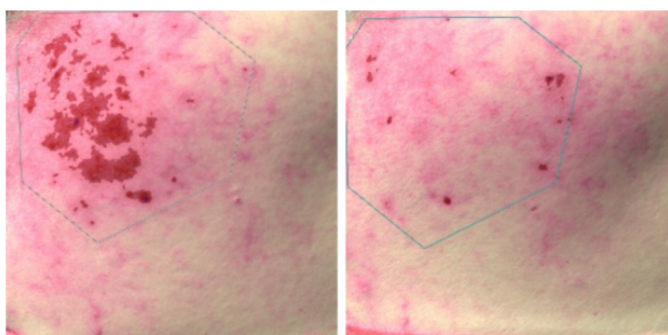
Note: compared with week 0, *means $P < 0.05$, **means $P < 0.01$.



Before the treatment
ultra-high concentration=5.262
affected area=54mm²

after28d the treatment
ultra-high concentration=0.006
affected area=0mm²

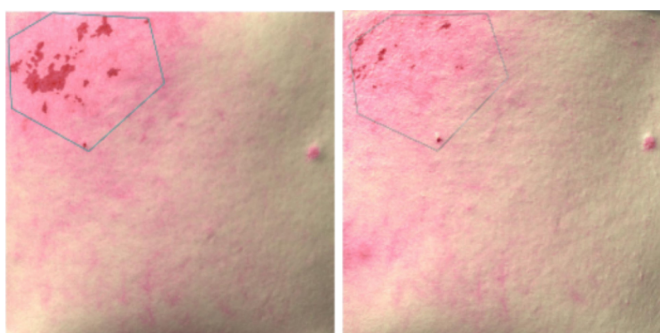
Figure 1. Volunteer 1.



Before the treatment
ultra-high concentration=18.832
affected area=199mm²

after28d the treatment
ultra-high concentration=4.788
affected area=12mm²

Figure 2. Volunteer 2.



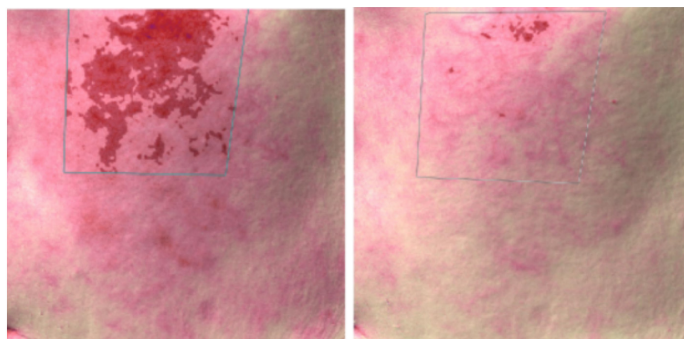
Before the treatment
ultra-high concentration=5.575
affected area=51mm²

after28d the treatment
ultra-high concentration=2.265
affected area=8mm²

Figure 3. Volunteer 5.

Clinical picture of ultra-high concentration of heme.

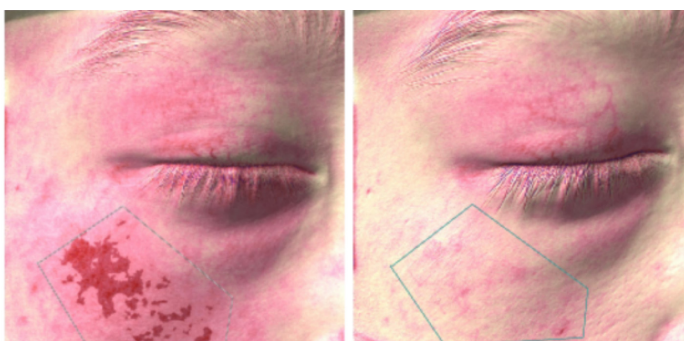
A total of 25 skin-sensitive volunteers were recruited. Antera3D imaging equipment was used to take pictures for 0 weeks (before treatment), and 13 volunteers showed skin damage and inflammation in heme (ultra-high concentration) mode. The heme (ultra-high concentration) data of the pictures of the 13



Before the treatment
 ultra-high concentration=27.314
 affected area=317mm²

after28d the treatment
 ultra-high concentration=2.358
 affected area=12mm²

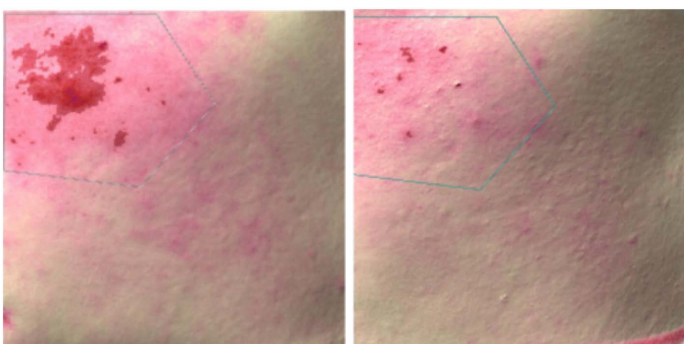
Figure 4. Volunteer 8.



Before the treatment
 ultra-high concentration=11.601
 affected area=119mm²

after28d the treatment
 ultra-high concentration=0.123
 affected area=0mm²

Figure 5. Volunteer 8.



Before the treatment
 ultra-high concentration=19.404
 affected area=143mm²

after28d the treatment
 ultra-high concentration=2.143
 affected area=6mm²

Figure 6. Volunteer 11.

volunteers at 0 weeks (before treatment and 4 weeks (28 days)) were compared, and the data of 20 damaged areas were obtained. The results showed that the product had the effect of skin repair and anti-inflammation after 4 weeks (28 days) of treatment. the data statistics are shown in **Table 5**.

Table 5. data statistics of heme (ultra-high concentration).

| Test time | 0 week | 4 week | Reduction rate % |
|-----------------------------------|-----------------|-------------------|------------------|
| Ultra-high concentration | 10.405 ± 8.978 | 3.449 ± 5.362** | 69.14% |
| Influence area (mm ²) | 75.058 ± 83.024 | 17.804 ± 34.259** | 77.47% |

Note: compared with 0 weeks, *means $P < 0.05$, **means $P < 0.01$.

It can be seen from **Table 5** that the ultra-high concentration of heme decreased by 69.14% and the area of the affected area decreased by an average of 77.47% after treatment for 4 weeks (28 days) and 0 weeks (before treatment) compared with 0 weeks ($P < 0.01$). The difference was statistically significant ($P < 0.01$). The human trial of ultra-high heme concentration test data show that the Camay series of cosmetics containing *Artemisia annua* extract can effectively repair sensitive skin, damaged skin and anti-inflammatory effects.

4. Conclusions

It is proved that CARE ARMOUR series cosmetics containing *Artemisia annua* extract have the effects of anti-inflammation, repairing skin barrier, repairing damaged skin and improving sensitive skin.

In the experiment of ear swelling in mice induced by xylene, it was found that the cosmetic samples containing *Artemisia annua* extract had good anti-inflammatory effects, and the data had a very significant statistical difference ($P < 0.01$). The swelling inhibition rates reached 60.40%, 73.36% and 74.01%, respectively, indicating that the cosmetics containing *Artemisia annua* extract has a good anti-inflammatory effect and can inhibit xylene-induced ear swelling in mice.

Human clinical trials showed that the TEWL value decreased after using CARE ARMOUR series cosmetics containing *Artemisia annua* extract. After 4 weeks of use, the TEWL value decreased by 21.51% compared with 0 week, with a significant statistical difference ($P < 0.05$). The hydration degree (A.U value) of the cuticle of the products containing *Artemisia annua* extract increased significantly after 1 week, 2 weeks and 4 weeks, in which the hydration degree of the cuticle of the right cheek increased by 63.90% in 1 week compared with 0 week, with a very significant statistical difference ($P < 0.01$). The hydration degree of horny layer in the right corner of the mouth increased by 90.32% in 4 weeks compared with 0 weeks, with a very significant statistical difference ($P < 0.01$). The results showed that the skin water loss through the epidermis decreased, the water content increased significantly, the skin barrier function was obviously repaired, and the condition of sensitive skin was improved.

Pictures were taken by Antera3D imaging equipment and analyzed in heme (ultra-high concentration) mode. After 4 weeks (28 days) of treatment with CARE ARMOUR series cosmetics containing *Artemisia annua* extract, compared with 0 week (before treatment), the ultra-high concentration of heme decreased by 69.14% on average, and the area of the affected area decreased by

77.47% on average, with a very significant statistical difference ($P < 0.01$). It shows that the use of CARE ARMOUR series cosmetics containing *Artemisia annua* extract can significantly repair the damage of skin barrier function, significantly reduce the symptoms of inflammation, reduce the degree of skin redness, and thus improve the condition of sensitive skin.

5. Discussion

The extensive amount of basic, preclinical and clinical research performed over the past decades has clearly demonstrated Artemisinin and its derivatives have anti-inflammatory properties and increase skin immunity [9] [17] [18] [19] [20] [21]. Researches show that Artemisinin can significantly reduce the expression of ROR- γ t, lower IL-6 and IL-17 levels, increase Foxp3 expression and promote Treg production, attenuate the phosphorylation activity of STAT3 [22]. The derivatives also have significant inhibitory effects on the production of IL-1, IL-2 and NO [18] [23].

The research of this paper includes that mouse ear swelling induced by xylene, the volunteers that the TEWL value, the hydration degree of the cuticle before and after using the product. The results are consistent with the previous reports, it is showed that containing *Artemisia annua* extract can significantly repair the damage of skin barrier function, significantly reduce the symptoms of inflammation, reduce the degree of skin redness, and thus improve the condition of sensitive skin.

Ethical Approval

Experiments were carried out in accordance with the ethical committee guidelines laid down by Institute of Chinese traditional medicine of Sichuan province regarding the care and use of animals for experimental procedures.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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