

Investigation of the Effects of Cosmetics on Skin Whitening and Anti-spot Efficacy Through Different Application Methods

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Abstract: To investigate the effects of two different application methods (combined use of toner, essence, lotion and alone use of essence) on the efficacy of whitening and anti-spot cosmetics. In this study the clinical trial was conducted to evaluate the difference of whitening, anti-spot efficacy between the combined use group and the alone use group by the spot size, density of pigmentary spots, contrast of isolated pigmentary spots, PIH (acne mark), and skin fairness. And the skin conditions were valuated by skin radiance, skin pore, skin evenness and global appearance of fine lines. The results of clinical trial showed that the improvement of spot size, density of pigmentary spots, contrast of isolated pigmentary spot, PIH (acne mark), skin fairness in combination group was significantly higher than that in alone use group. Furthermore, the two different application methods also illustrated the same trend for changes in skin conditions including skin radiance, skin pore, skin evenness and global appearance of fine lines. These findings indicate that the whitening, anti-spot efficacy and skin conditions improvement of combined use of toner, essence and lotion was better than that of use essence alone.

1. Introduction

The pursuit of fair skin has long been a cultural ideal for women in Eastern societies, leading to the popularity of whitening and anti-spot cosmetics among this demographic. Melanin is one of the primary factors contributing to skin pigmentation^[1].

Melanin is synthesized by melanocytes located in the basal layer of the epidermis^[2] and subsequently transported to nearby keratinocytes^[3, 4]. As keratinocytes mature, melanin gradually migrates from the basal layer towards the stratum corneum before ultimately being shed. Additionally, it has been reported that autophagy can contribute to melanin degradation^[5]. Therefore, the synthesis, transport and metabolism of melanin are represent crucial biological mechanisms for the development of whitening and anti-spot cosmetics.

In this study, a range of cosmetics was developed with efficacy in skin whitening and anti-spot. The product line includes toner, essence, and lotion, with the main active ingredients being salicylic acid, lipohydroxy acid, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), phenylethyl resorcinol, ferulic acid, niacinamide and ectoin. Salicylic acid, lipohydroxy acid and HEPES all have the effect of promoting exfoliation. Salicylic acid as a peeling agent has a number of indications, including acne vulgaris, melasma, photodamage, freckles, and lentigines^[6]. Lipohydroxy acid acts on the desmosomes between keratinocytes, causing cell separation and promoting desquamation^[7]. HEPES is a safe and efficient osmolyte and stimulates protease activity in the stratum corneum, promoting desquamation^[8]. Phenylethyl resorcinol exhibits a potent inhibitory effect on tyrosinase and is frequently employed as a whitening agent in cosmetic formulations^[9]. Ferulic acid is a free radical scavenger, but also an inhibitor of enzymes that catalyze free radical generation and an enhancer of scavenger enzyme activity. Moreover, ferulic acid has been demonstrated to exert inhibitory effects on melanin biosynthesis^[10-11]. Niacinamide inhibits melanin transport to keratinocytes, thereby reducing skin pigmentation. It is a widely used whitening agent in cosmetics^[12]. Ectoin, a cyclic amino acid derivative widely present in halophilic bacteria, streptococcus and other non-halophilic bacteria, exhibits excellent photoprotective effects

such as delaying skin aging, UV protection, antioxidant activity, moisturization and anti-inflammatory properties^[13].

In summary, this range of cosmetics can play a whitening and anti-spot effect by accelerating melanin metabolism through promoting cuticle exfoliation, inhibiting tyrosinase to decrease melanin synthesis, impeding melanosome transport, and possessing anti-oxidation properties.

In order to compare the effect of different application methods of this range of cosmetics on whitening, anti-spots and improvement of skin conditions efficacy, the study was conducted through clinical trial, which provided experimental basis for the application and development of whitening and anti-spot cosmetics, as well as provided reference for a comprehensive and effective evaluation of the efficacy of whitening and anti-spot skin care products.

2. Experimental section

2.1 Reagents and instruments

Reagents and instruments Toner, essence, lotion, standard facial cleanser, standard moisturizer, standard sun protection were all provided by L'Orea; VISIA-CR(Canfield) .

2.2 Clinical trial

2.2.1 Volunteers recruitment

Ninety-two Chinese women meeting the inclusion criteria were recruited, aged 20 to 45 years old and with all skin types, including those who self-declared as having sensitive skin. All individual subjects sign an informed consent before the study and agree to comply with all requirements outlined of the study protocol.

According to the dermatologist's assessment, the volunteer must meet the following criteria for facial features: at least one isolated spot (minimum 3mm) surrounded by a clear area; density of pigmentary spots (grade \geq 2, Skin Aging Atlas^[14]); at least one visible non-inflammatory acne mark; contrast of isolated pigmentary spot on face (grade \geq 2, Skin Aging Atlas); and skin fairness ($2\leq$ grade \leq 6, modified Griffiths 10-point scale (0-9)^[15]).

2.2.2 Clinical trial process

The volunteers were eluted according to the standardized method and skincare product. The test was started after the washout period.

The volunteers were randomly divided into two groups, 48 in the alone use group and 46 in the combined use group. In addition to standard skincare products, the alone use group exclusively applies essence while the combination use group incorporates toner, essence and lotion. After 20 minutes of constant temperature and humidity environment balance, the volunteers' faces were evaluated by dermatologist and photographs were taken with the instrument, obtain baseline data.

After 2, 4, and 8 weeks of sample usage, the volunteers underwent VISIA-CR® imaging to evaluate skin condition. The size of tracking spots was measured using a skin ruler, while a dermatologist evaluated density of pigmentary spots, contrast of isolated pigmentary spot, Pigmentation in a single acne mark, skin fairness, skin radiance, skin pore, skin evenness and global appearance of fine lines utilizing the Skin Aging Atlas and modified Griffiths scale (0-9).

2.3 Statistical analysis

Statistical analysis and mapping were performed using GraphPad Prism 8.02. The results of the clinical trial were presented as Mean \pm SEM using the Wilcoxon Signed Ranks test. A significance level of $P < 0.05$ was adopted to indicate statistical significance, while a more stringent threshold of $P < 0.01$ was used to denote high statistical significance. Compared with the alone use group, significance is represented as *, $P < 0.05$ is indicated by *, and $P < 0.01$ is indicated by **.

3. Results and discussion

3.1 Size of isolated an spot

Isolated spots were selected on both sides of the volunteers' faces and measured using a skin ruler before and after 2, 4, and 8 weeks of cosmetics use. The spot size in the alone use group decreased by 3.11%, 4.18%, 8.22% at each time point, while the combined use group showed an even greater decrease of 11.47%, 15.21%, 19.41% from baseline after 2, 4, and 8 weeks of cosmetics use (Fig 1). Statistical analysis indicated that the combination group exhibited a significantly greater reduction in spot size after 2, 4, and 8 weeks of cosmetic use compared to the alone group ($P < 0.01$). These findings suggest that combined application yielded superior improvements in volunteer's spot size relative to using essence alone. As shown in Fig. 1.

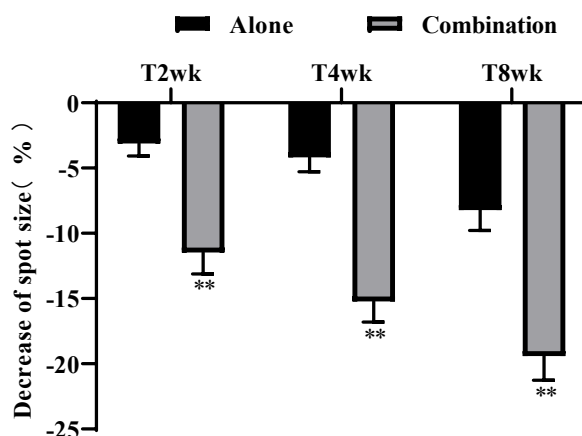


Fig.1 The decrease of isolated spot size.

3.2 Density of pigmentary spots

The Skin Aging Atlas utilized a scoring system ranging from 0 to 7 to evaluate the density of facial pigmentary spots in volunteers before and at 2, 4, and 8 weeks following cosmetic application. The density of pigmentary spots in the alone use group decreased by 8.39%, 10.65%, 26.06%, respectively at 2, 4, and 8 weeks from the baseline, and that in the combined use group decreased by 15.77%, 22.86%, 33.46% (Fig 2). Statistical analysis revealed that the combination group exhibited a significantly higher reduction in pigmentary spots density at 2 and 4 weeks compared to the alone use group ($P < 0.05$). Although there was no significant difference between the two groups after 8 weeks, the combined use still resulted in a higher reduction than alone use group. The results suggest that combining cosmetics use is more effective than using essence alone in improving the density of pigmentary spots. As shown in Fig. 2.

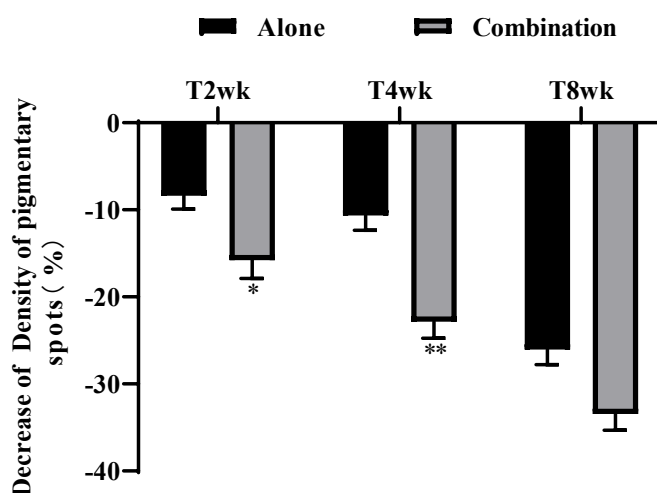


Fig.2 The decrease of density of pigmentary spots.

3.3 Contrast of isolated pigmentary spot

According to the Skin Aging Atlas, the volunteers' facial Contrast of isolated pigmentary spot was assessed using a scoring system ranging from 0 to 5 before and at 2, 4, and 8 weeks after cosmetics application. The contrast of isolated pigmentary spot in the alone use group decreased by 7.04% ,6.91%, 19.23% compared with baseline, while it decreased by 23.22%, 29.93%, 35.22% compared with baseline in the combined use group, after 2, 4, 8 weeks (Fig 3). Statistical analysis showed that the combined use group demonstrated a significantly greater reduction in contrast of isolated pigmentary spot after 2, 4, and 8 weeks of cosmetic usage compared to the alone use group ($P < 0.01$). These findings suggest that combining cosmetics is more effective than using essence alone for improving facial contrast of isolated pigmentary spots. As shown in Fig. 3.

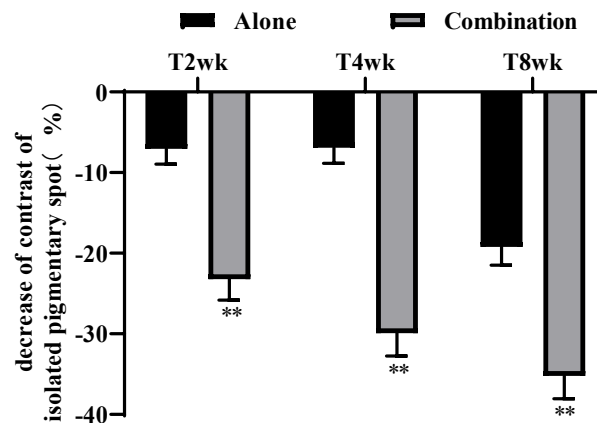


Fig.3 The decrease of contrast of isolated pigmentary spot.

3.4 Pigmentation in a single acne mark

The facial pigmentation in a single acne mark of the volunteers were evaluated using the modified Griffiths 0 to 9-point scale at baseline and 2, 4, and 8 weeks cosmetics use.

At 2, 4, and 8 weeks, the alone use group showed 8.39%, 14.10%, 24.78% reduction in pigmentation of a single acne mark compared with baseline respectively, while the combined treatment group exhibited a greater decrease of 38.12%, 56.34%, 71.56% from baseline (Fig 4). Statistical analysis indicated that the combined use group exhibited a significantly greater reduction in pigmentation of a single acne mark after 2, 4, and 8 weeks of cosmetics usage compared to the alone use group ($P < 0.01$). These findings suggest that combination treatment is more effective than use essence alone for improving pigmentation of a single acne mark on the face of volunteers. As shown in Fig. 4.

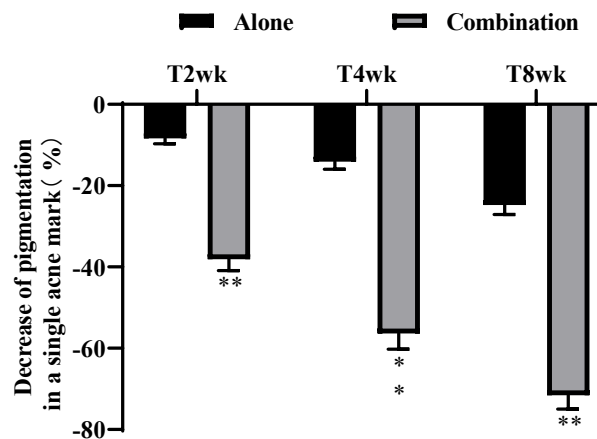


Fig.4 The decrease of pigmentation in a single acne mark.

3.5 Skin fairness

The facial skin fairness of the volunteers were evaluated using the modified Griffiths 10-point

scale (0-9) at baseline and at 2, 4, and 8 weeks cosmetics use, a lower score on this scale indicates a fairer complexion.

The skin fairness of the alone use group was showed 4.14%, 8.25%, 16.80% reduction in skin fairness compared with baseline respectively, at 2, 4 and 8 weeks, while it decreased by 12.90%, 20.80%, 25.33% in the combination use group (Fig 5). Statistical analysis showed that after 2, 4 and 8 weeks of using the cosmetics, the improvement of skin fairness in the combination use group was significantly higher than that in the alone use group ($P < 0.05$). It can be inferred that combination treatment is more effective than alone use essence for improving facial skin fairness of volunteers. As shown in Fig. 5.

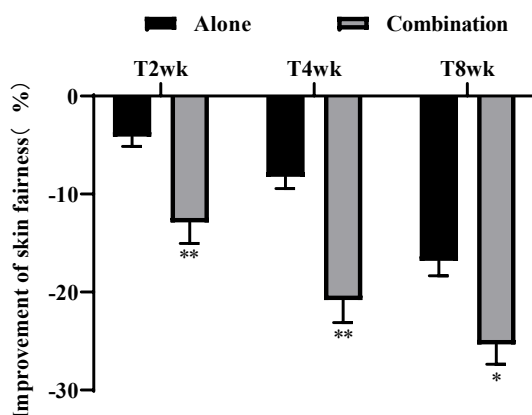


Fig.5 The improvement of skin fairness.

3.6 Improvement of skin condition

The facial skin conditions, including skin radiance, skin pore, skin evenness and global appearance of fine lines of the volunteers, were assessed using the modified Griffiths 10-point scale (0-9) at baseline and at 2, 4, and 8 weeks cosmetics use, a lower score on this scale indicates better skin conditions.

The skin radiance of the alone use group was showed 10.86%, -18.17%, 25.35% reduction in alone use group compared with baseline at 2, 4 and 8 weeks respectively, while it decreased by 23.95%, 30.35%, 31.52% in the combination use group (Fig. 6A). Statistical analysis showed that the improvement of skin radiance in the combination use group was significantly higher than that in the alone use group ($P < 0.05$) after 2, 4 weeks of using the cosmetics. Although there was no significant difference between the two groups after 8 weeks, the combined use still resulted in a higher reduction than alone use group. The data indicated that combination treatment is more effective than alone use essence for improving facial skin radiance of volunteers.

The skin pore of the alone use group was showed 4.87%, 11.24%, 25.35% reduction in alone use group compared with baseline at 2, 4 and 8 weeks respectively, while it decreased by 14.36%, 19.26%, 21.09% after 2, 4 and 8 weeks of using the cosmetics (Fig. 6B). Statistical analysis showed that the improvement of skin pore in the combination use group was significantly higher than that in the alone use group ($P < 0.01$) after 2, 4 and 8 weeks of using the cosmetics. It indicated that combination treatment is more effective than alone use essence for improving facial skin pore of volunteers.

The skin evenness of the alone use group was showed 2.31%, 6.24%, 13.71% reduction in alone use group compared with baseline at 2, 4 and 8 weeks respectively, while it decreased by 8.77%, 15.07%, 21.40% after 2, 4 and 8 weeks of using the cosmetics (Fig. 6C). Statistical analysis showed that the improvement of skin evenness in the combination use group was significantly higher than that in the alone use group ($P < 0.01$) after 2, 4 and 8 weeks of using the cosmetics. It indicated that combination treatment is more effective than alone use essence for improving facial skin evenness of volunteers.

The global appearance of fine lines of the alone use group was showed 14.92%, 19.31%, 26.85% reduction in alone use group compared with baseline at 2, 4 and 8 weeks respectively, while it

decreased by 38.22%, 42.63%, 58.77% after 2, 4 and 8 weeks of using the cosmetics(Fig. 6D). Statistical analysis showed that the improvement of global appearance of fine lines in the combination use group was significantly higher than that in the alone use group ($P < 0.01$) after 2, 4 and 8 weeks of using the cosmetics. It indicated that combination treatment is more effective than alone use essence for improving facial global appearance of fine lines of volunteers. As shown in Fig. 6.

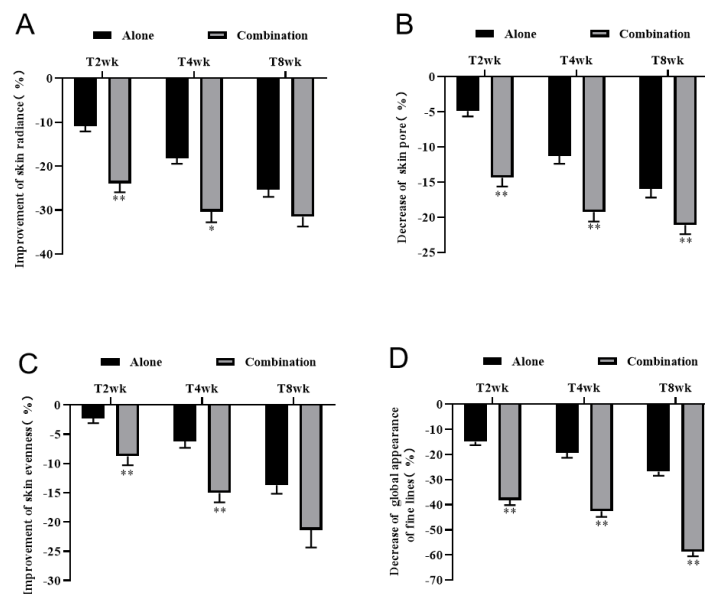


Fig. 6 Improvement of skin conditions. A: Improvement of skin radiance. B: Decrease of skin pore. C: Improvement of skin evenness. D: Decrease of global appearance of the fine line.

4. Conclusion

This study investigated the impact of various application methods on the efficacy of cosmetics in terms of skin whitening, anti-spot and improvement skin conditions using clinical trial.

Systematic evaluation and statistical analysis revealed that the combined use of toner, essence and lotion for 2, 4, and 8 weeks resulted in a significantly higher improvement in spot size, density of pigmentary spots, contrast of isolated pigmentary spot, pigmentation in a single acne mark, skin fairness and skin conditions compared to the group using only essence. The results indicate that the whitening, anti-spot efficacy and skin conditions improvement of combined use of toner, essence and lotion was better than that of use essence alone. Additionally, this study evaluated facial spot from a multi-parameter system, providing a reference method for the efficacy evaluation of whitening and anti-spot cosmetic interventions.

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