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Original Article

Safety and efficacy assessment of skin gel containing nanoemulsion of *Phyllanthus emblica* extract: A randomized, double-blind, placebo-controlled study

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Abstract

A skin gel containing a nanoemulsion of *Phyllanthus emblica* branch extract (emblica nanogel) was tested for its safety and efficacy in a randomized, double-blind, placebo-controlled clinical trial. The patch skin irritation test showed that neither the emblica nanogel nor the placebo nanogel caused skin erythema. The subjects receiving emblica nanogel had a significantly lower melanin index of the cheek at weeks 2, 4, 6, and 8, of the forehead at weeks 4, 6, and 8, and of the forearm at week 8 compared to the placebo group. Skin elasticity was observed to increase for the cheek at week 4, forehead at week 6, and there was no change for the forearm. The levels of skin moisture and erythema of the subjects were not significantly different from the base line levels. In conclusion, the application of emblica nanogel resulted in significant skin whitening during the 8 weeks of application.

Keywords: skin whitening, emblica, nanoemulsion, randomized double-blind, clinical trials, melanin index

1. Introduction

Healthy and youthful skin is desirous for people of all ages, especially those in the growing aged population. With increasing age, skin undergoes alterations characterized by a loss of elasticity and moisture content, uneven tone, and a tendency to develop wrinkles. Photoaging is very common and causes not only increased melanogenesis but also a reduction in the amount of collagen, eventually giving rise to increased skin pigmentation and wrinkles (Baroni *et al.*, 2012; Gordon, Mansur, & Gilchrest, 1989; Romero-Graillet *et al.*, 1996). Several skin care products have been introduced following the demand for lightening the skin color and looking younger and the most common route of administration is a topical application. Many melanogenesis inhibitors such as hydroquinone, arbutin, retinoic acid, kojic acid, and α–hydroxy acids have been used as whitening agents in cosmetic products although there are often some side effects (Griffiths *et al.*, 1993; Haddad *et al.*, 2003; Maeda & Fukuda, 1996; Mishima, Ohyama, Shibata, Seto, & Hatae, 1994; Stiller *et al.*, 1996). During the past several decades, plant extracts have been studied in the search for natural products with anti-tyrosinase activity. These plant extracts may offer a natural bioactive source for a broad spectrum of compounds including polyphenolics and flavonoids (Chen, Wei, & Marshall, 1991; Kubo *et al.*, 2000; Parvez *et al.*, 2006)

Emblica (*Phyllanthus emblica* L.), (Euphorbiaceae) is widely distributed in the subtropical and tropical areas. The fruit is used as a major constituent of various traditional and Ayurvedic medicines. Following reports on the pharmacological effects of emblica fruit, which include antioxidant (Liu, Zhao, Wanga, Yangb, & Jiang, 2008), anti-inflammatory (Muthuraman, Sood, & Singla, 2011), and protection from UV-B-induced photo-aging (Adil *et al.*, 2010), its demand as an ingredient in cosmetic products has increased. However, the supply is limited according to the size of the annual crop. The alternative use of other parts of emblica as potential substitutes for the fruit is desirable. Gallic acid is a major phenolic compound in the emblica fruit. Previous reports have demonstrated the potential of the branches and stems of *P. emblica*

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as alternative sources of the phenolic compounds including catechin, epicatechin gallate, epigallocatechin (EGC), epigallocatechin gallate (EGCG) and gallocatechin (Balasundram, Sundram, & Samman, 2006; Dufresne & Farnworth, 2003). Our previous data indicated high amounts of several phenolic compounds including ascorbic acid, gallic acid, catechin, vanillic acid, vanillin, ferulic acid, and ellagic, EGC, EGCG in the alcoholic extract of *P. emblica* branches (Sripanidkulchai & Junlatat, 2014).

P. emblica branch extract also exhibited potent inhibitory effects on mushroom tyrosinase activity and down regulation of tyrosinase related proteins genes in B16 murine melanoma cells (Sripanidkulchai & Junlatat, 2014). With the advantage of enhanced solubility, bioavailability, and stability of nanocarrrier dosage forms, a nanoemulsion of *P. emblica* branch extract was successfully developed with sustainable releases of EGC and EGCG (Chaiittianan & Sripanidkulchai, 2014). Therefore, in this study the nanoemulsion of emblica branch extract was further developed as a skin gel and tested for its safety and efficacy.

2. Materials and Methods

2.1 Preparation of skin gel products

P. emblica collected from Amphur Muang in Khon Kaen Province, Thailand was dried at 50 °C and ground to a powder, then extracted with 50% ethanol and filtered through Whatman® No.1 paper. The filtrate was dried using a rotary evaporator at 45 °C, and then freeze-dried in a lyophilizer with a yield of 5.4%.

Under high-performance liquid chromatography analysis, the extract contained several phenolic compounds that included three major compounds: vanillic acid, galic acid, and epigallocatechin at concentrations of 6.9, 6.3, and 1.9 mg/g, respectively. A nanoemulsion containing the ethanolic extract of *P. emblica* branch (0.15%), isopropyl myristate (0. 6%), Brij® (0.35%), and distilled water (98.9%) was prepared by a modified microemulsion technique with hot high pressure homogenization as previously described by Chaiittianan and Sripanidkulchai (2013). Then the skin gel (so-called emblica nanogel) was formulated for a composition of nanoemulsion (80%), hydroxyethylcellulose base (2%), glycerine (3%), propylene glycol (0.2%), methylparaben (0.04%), propyl paraben (1.76%), and distilled water (13%). Finally, each gram of emblica nanogel contained 1.2 mg of *P. emblica* extract. The placebo gel (so-called placebo nanogel) contained the same pharmaceutical ingredients, except for the ethanolic extract of *P. emblica*.

2.2 Clinical studies

The randomized, double–blind, placebo-controlled protocol was approved by Khon Kaen University Ethical Committees on clinical trials (HE532270). The demographic data of the participants are shown in Table 1.

2.3 Skin irritation test

Prior to product application, a patch skin irritation test was conducted in 50 healthy Thai volunteers who resided in Khon Kaen Province. They were informed about the study conditions and each subject signed an informed consent form. Apart for the absence of skin disease and no current use of medication, no specific exclusion criteria were used. All subjects applied 0.5 mL each of the test materials in an area measuring 1×1 cm on the upper right arm. Our test products included (1) distilled water (negative control), (2) 1% sodium lauryl sulfate (positive control), (3) emblica nanogel, and (4) placebo nanogel. There were two rows of treated areas, the first started 4 cm from the shoulder, and the second spaced 0.5 cm from the first. After product application, clean cotton patches and clear plastic tape were used to cover each area. After 24 h, signs of skin irritation were assessed by evaluating the degree of skin redness and swelling with the naked eye and were given scores at 5 levels as 0 (no erythema), 1 (very slight erythema), 2 (well-defined erythema), 3 (moderate to severe erythema), and 4 (injury in depth) (North American Science Association, nd). In parallel, the erythema index was also measured colorimetrically using a Mexameter® (MX 18, Courage & Khazaka, Germany).

2.4 Efficacy test

2.4.1 Subjects and treatment

Forty healthy volunteers were separately included in the study after their informed consent was obtained. The exclusion criteria were: having a skin disease, taking medication or food supplements, using whitening products, allergy to facial products, and pregnancy or lactation. In this double-blind trial with a placebo control, the subjects were randomly assigned into two groups by a blind investigator. Two sets of products with identical packages were blindly labeled to be number one and number two. Each subject received two identical packages and was instructed to daily and separately apply these two products (either emblica nanogel or placebo nanogel) on the assigned side of face (0.3 g of gel on half face) and forearm (0.15 g of gel on the area of 5×5 cm) in the morning and the evening. The trial lasted for 8 weeks. The subject and product codes were opened after completion of the experiment.

2.4.2 Assessment of product efficacy

The subjects were evaluated for the product efficacy by assessing three different areas of the skin, which were check, forehead, and forearm for 4 items, including melanin index and erythema (Maxameter® MX18, Courage & Khazaka, Germany), moisture and elasticity (Multi Dermascope® MDS800, Courage & Khazaka, Germany) at 0, 2, 4, 6, and 8 weeks after application of the product. The measurements of each point were conducted three times by a blind investigator and the average value was used for further analysis.

Table 1. Demographic information of the participants.

Factors	Irritation test (n=49)	Efficacy test (n=39)
Sex		
Male, n (%)	10 (20.41)	5 (12.82)
Female, n (%)	39 (79.59)	34 (87.18)
Age, y, mean (range)	28.54 (20–52)	36.8 (22–52)

2.5 Statistical analysis

Data are expressed as mean±SD. Significance of differences were examined using one-way analysis of variance (ANOVA) and Duncan's multiple range test. Significant differences were set at P-values less than 0.05.

3. Results

3.1 Product safety

After one subject dropped out, the skin irritation test included 49 healthy volunteers (10 males and 39 females) with a mean age of 28.54 (range 20–52) years. Out of the 49 volunteers, 92% had never reported an allergy to any substance. At 24-h post-application and using a naked eye evaluation, the nanogel products caused negligible irritation to the skin. No erythema was found in 93.9% of the subjects at the applied area of the upper arm and only 6.1% of subjects showed very slight erythema. The positive control (1% sodium lauryl sulfate) caused very slight erythema in 8.2% and well-defined erythema was found in 91.8% (Figure 1). This was confirmed in measurements with the Mexameter where the 1% sodium lauryl sulfate caused significant skin erythema (Figure 2). The results suggested that the nanogel products were safe to use in further studies.

3.2 Product efficacy

Forty subjects were separately recruited into the study and were subsequently randomized into two different treatments on their body. Twenty subjects were asked to blindly apply product number one (emblica nanogel) on the right half of the face and forearm, and product number two (placebo nanogel) on the left half of the face and forearm, and *vice versa* for another 20 subjects. After one subject dropped out, 39 subjects (5 males and 34 females) with a mean age of 36.8 (range 22–52) years completed the study on product efficacy without adverse effects.

Compared to the placebo nanogel, the emblica nanogel significantly decreased melanin index at weeks 2, 4, 6, and 8 after application to the cheek (P<0.001). In parallel, the melanin indices of the forehead in the emblica nanogel treated subjects had significantly decreased at weeks 4, 6, and 8 (P< 0.001), whereas the melanin indices of the forearm had significantly decreased only at week 8 (P<0.001) (Figure 3). However, the melanin index of emblica nanogel treatments at the cheek, forehead, and forearm showed lower values that were gradual but significant compared to the baseline at all time points in this study (P<0.05).

The emblica nanogel significantly increased skin elasticity of the cheek at week 4 and of the forehead at week 6. However, the skin elasticity of the forearm did not change (Figure 4). Compared to the baseline data, the emblica nanogel decreased elasticity of the forehead at week 8 and increased elasticity of the forearm at weeks 2, 4, and 6 of the study. There were no significant differences in skin moisture between the test and placebo groups (Figure 5). However, compared to the baseline data both the emblica nanogel and placebo nanogel groups tended to have increased skin moisture. In terms of erythema index, which reflects the degree of skin irritation, both tests and placebo products tended to decrease the erythema index (Figure 6). Finally, a whitening effect of the emblica nanogel was demonstrated (Figure 7).

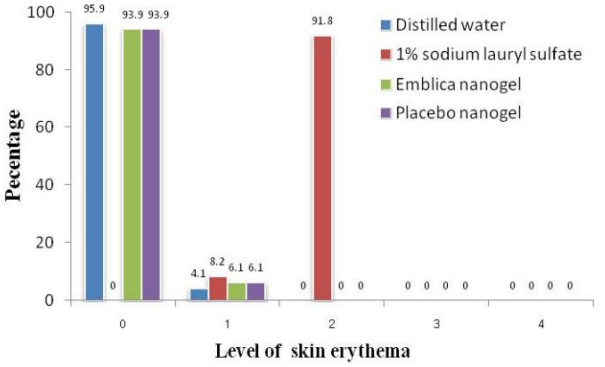

Figure 1. Evaluation of skin appearance (by naked eye) at 24-h post-application of emblica nanogel. Data obtained from 49 volunteers (10 males and 39 females) with an average age of 28.5 (range 20–52) years. (0 = no erythema, 1 = very slight erythema, 2 = well defined erythema, 3 = moderate to severe erythema, 4 = injury in depth)

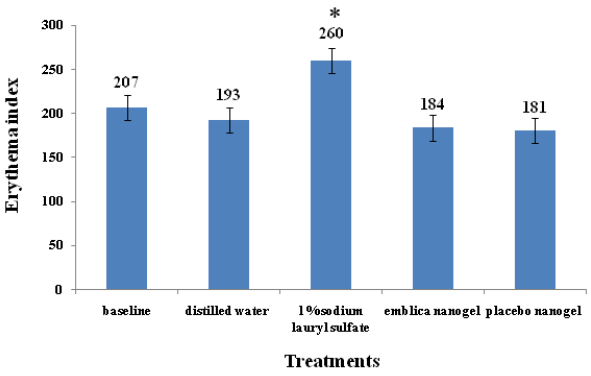

Figure 2. Erythema indices of 49 volunteers' arms at 24-h post-application of emblica nano products. *significant difference from baseline at P<0.05

4. Discussion and Conclusion

Measuring skin irritation potential is necessary as part of toxicological evaluation of cosmetic products, and in this study it was conducted prior to the efficacy test. The emblica nanogel containing emblica branch extract as the main ingredient and placebo nanogel containing other ingredients of gel base caused no skin irritation. There were no adverse effects such as burning or pruritis. Therefore, emblica and placebo nanogels were considered safe for use in subjects for the efficacy test.

In this study we demonstrated the potential of a nanoemulsion containing *P. emblica* branch extract formulated into a nanogel form as a skin whitening agent with additional benefits. The emblica nanogel displayed skin whitening effects from the second week of product application and throughout the 8 weeks of the study period. Based on the different degree of pigmentation of the skin; therefore, three areas were included in this study. The melanin index of emblica nanogel treated cheek skin was most sensitive and lower

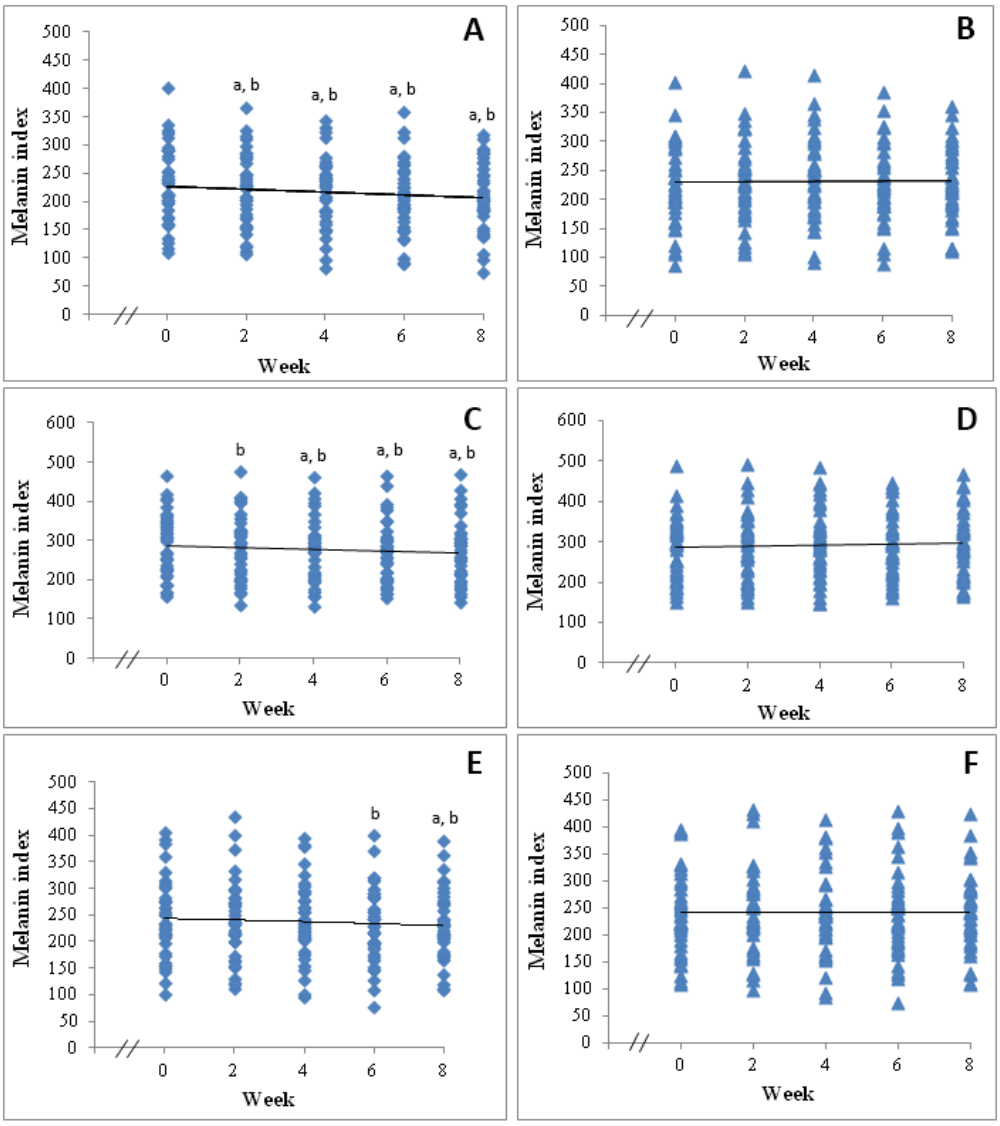

Figure 3. Melanin indices of volunteers' skin treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm), [a] significant difference from placebo during the same week at $P<0.05$, [b] significant difference from week 0 within the same group at $P<0.05$

than those of the placebo nanogel treated from the second week, whereas the melanin indices of the emblica nanogel applied to the forehead and forearm were affected at week 8. In contrast, the melanin indices of placebo nanogel treatments of these three areas were unchanged throughout the 8-week period. Our results confirm previous reports on the antioxidant and anti-tyrosinase activities of *P. emblica* branch extract as an active ingredient in the emblica nanogel (Sripanidkulchai & Junlatat, 2014). In terms of the elasticity index, compared to the placebo group, the emblica nanogel significantly increased the elasticity at the cheek only at 4 weeks of treatment which suggested a mild effect of the emblica nanogels on skin elasticity. Both the emblica and placebo nanogels showed a tendency to increase the moisture index of the cheek, forehead, and forearm from the baseline values which probably indicated the moisturizing effect of the gel base of these products. The erythema index that reflects skin irritation demonstrated that both the emblica and placebo nanogels did not irritate the skin of the subjects throughout the 8-week applications. Moreover, compared to the baseline values (at week 0), a significant decrease in erythema index was observed at 2 and 8 weeks for the cheek, at 4 and 8 weeks for the forehead, and at 8 weeks for the forearm. These results indicated the skin lightening effect of the products.

Several phenolic compounds have been reported to be among the chemical constituents of emblica branch extract that include gallic acid, vanillic acid, epigallocatechin, epigallocatechin gallate, and ellagic acid (Chaiittianan & Sripanidkulchai, 2014). These naturally occurring polyphenolic compounds have previously been found to inhibit melanogenesis. Gallic acid significantly inhibited melanin synthesis and tyrosinase activity and decreased the expression of

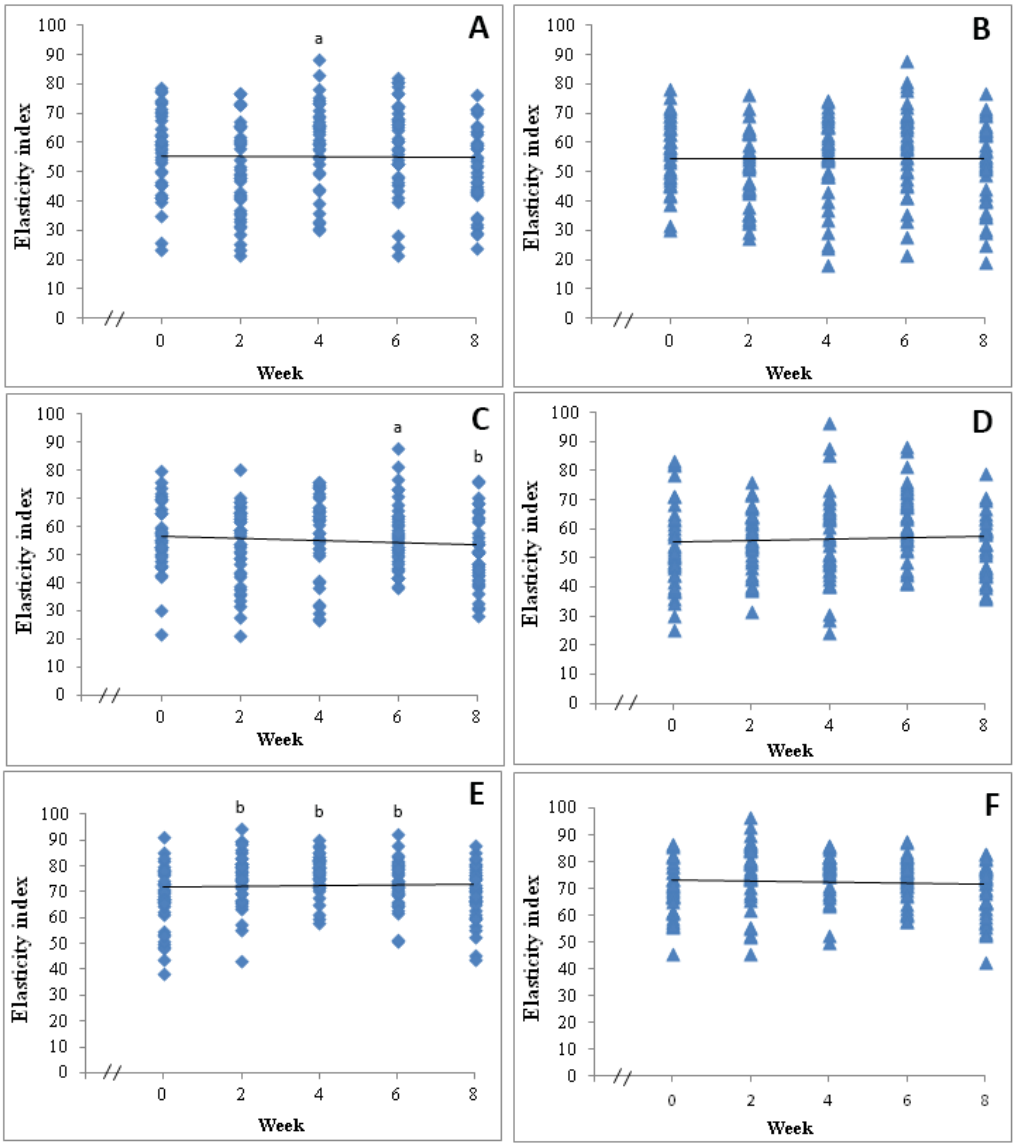

Figure 4. Elasticity indices of volunteers' skin treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm),[a] significant difference from placebo during the same week at $P<0.05$, [b] significant difference from week 0 within the same group at $P<0.05$.

melanogenesis-related proteins, such as microphthalmia-associated transcription factor, tyrosinase, tyrosinase-related protein-1, and dopachrome tautomerase via several markers of the signal cascade pathway, including the activation of the MEK/ERK and PI3k/Akt signaling pathways (Kumar *et al.*, 2013; Su *et al.*, 2013). Ellagic acid was reported to inhibit melanogenesis (Phrutivorapongkul *et al.*, 2013; Shimogaki, Tanaka, Tamai, & Masuda, 2000). Extracts from several parts of plants containing these phenolic compounds have been shown to have potential as whitening cosmetic products based on their anti-tyrosinase activities which include grape seeds and peels (Hsu *et al.*, 2012), peaches (Kim, Kim, Yu, & Yook, 2012), fruit pericarp of *Lichi chinensis* (Kanlayavattanakul, Ospondpant, Ruktanonchai, & Lourith, 2012), pomegranate fruit peel (Fawole, Makunga, & Opara, 2012), mushrooms (Alam, Yoon, Lee, Lee, & Lee, 2011; Yoon *et al.*, 2011), and green tea (No *et al.*, 1999). Therefore, *P. emblica* branch extract can be an alternative source of these phenolic compounds. Taken together the data from this study suggest that nano-emblica gels have skin lightening effects without side effects or skin irritation.

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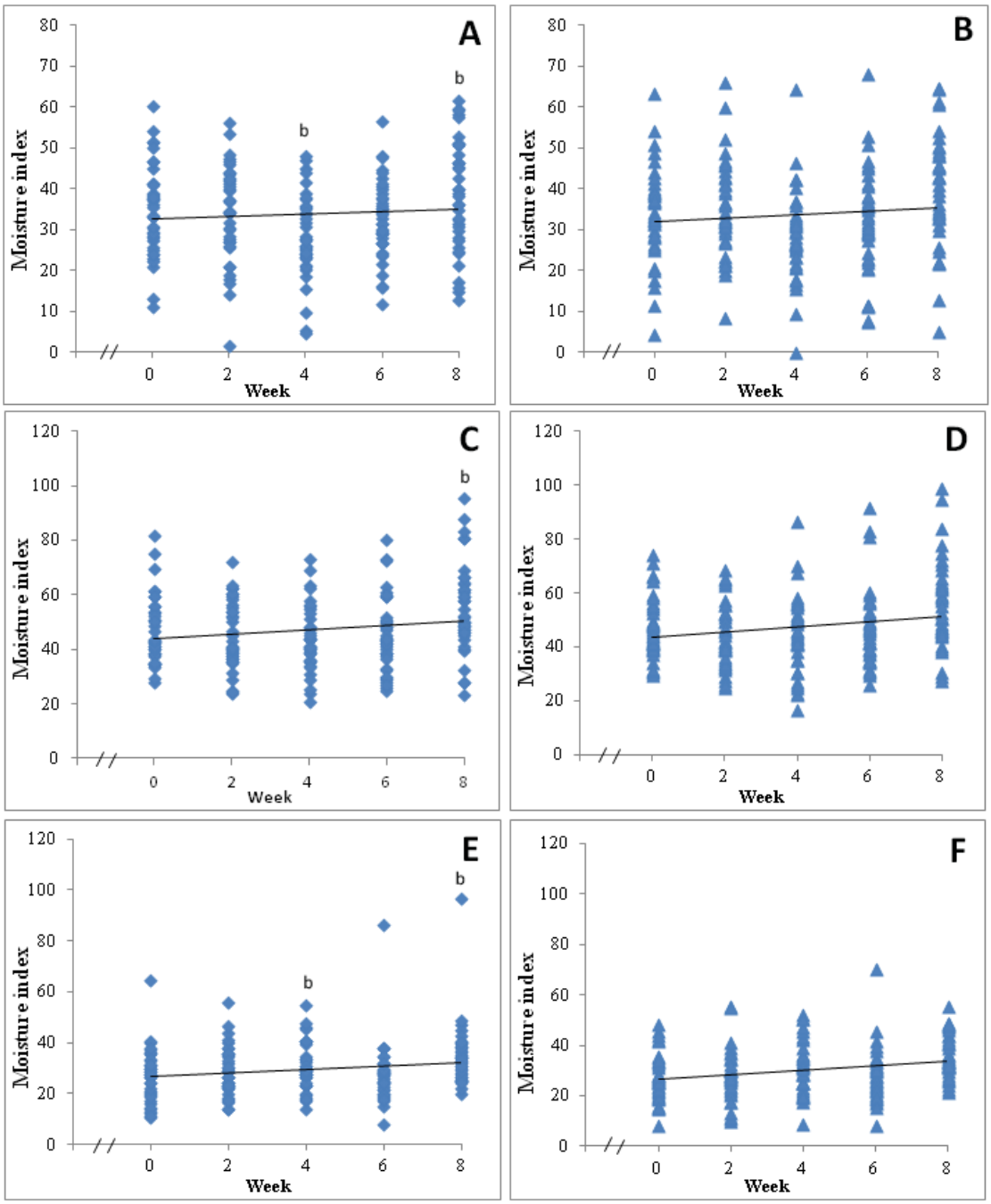

Figure 5. Moisture indices of volunteers' skin treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm), [b] significant difference from week 0 within the same group at $P<0.05$.

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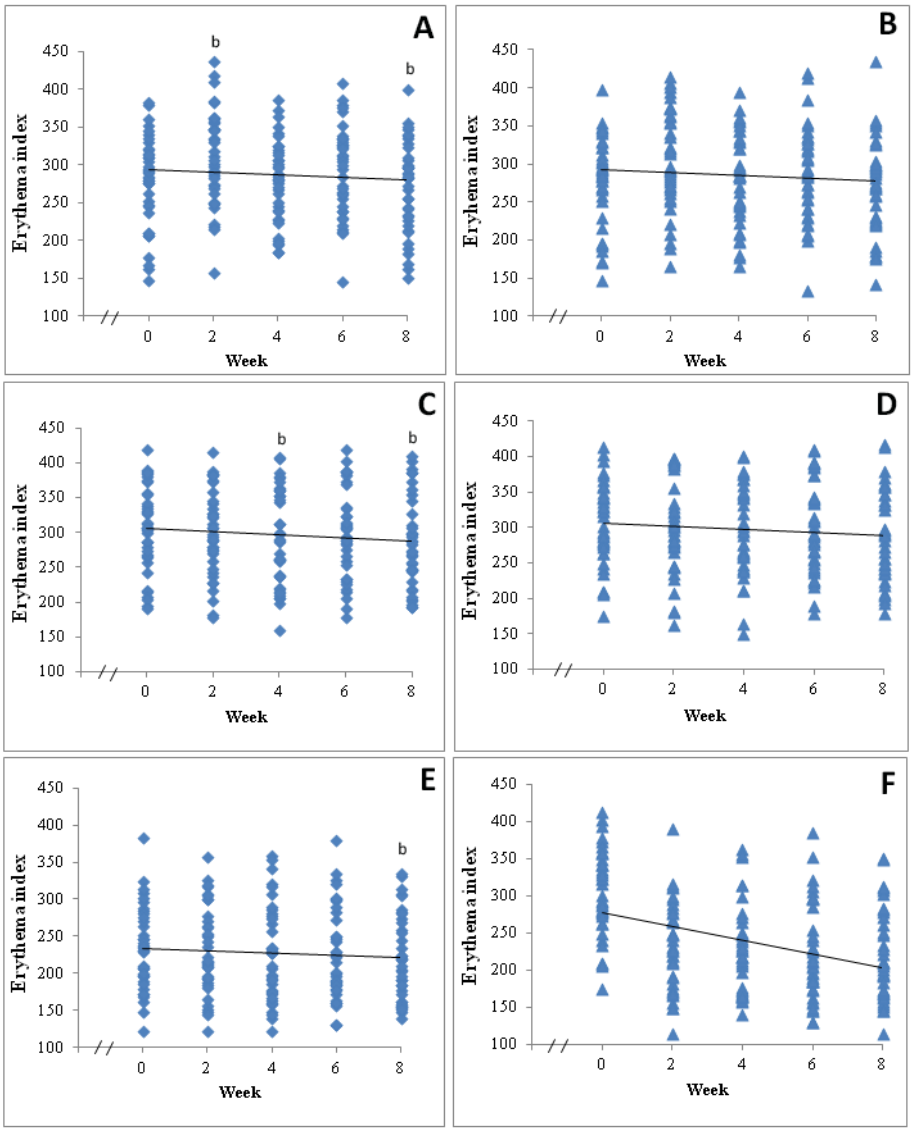

Figure 6. Erythema indices of volunteers treated with emblica nanogel (A = cheek, C = forehead, E = forearm) and placebo nanogel (B = cheek, D = forehead, F = forearm), [a] significant difference from placebo during the same week at P<0.05, [b] significant difference from week 0 within the same group at P<0.05.

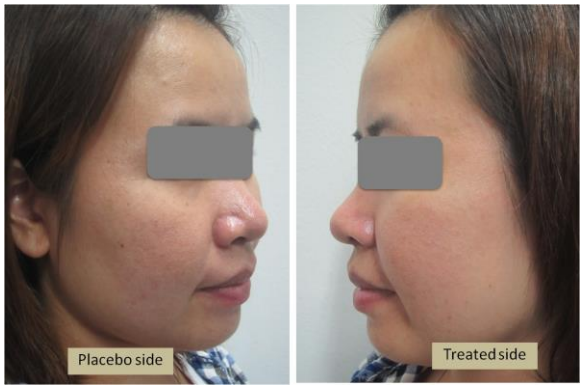

Figure 7. Whitening effect of the skin gel product on the face of a representative volunteer.

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