Comparative Study on Depigmenting Agents in Skin of Color

by ACHALA LIYANAGE, MD; GAYANI LIYANAGE, PhD; GANGA SIRIMANNA, MD, MRCP and NANNA SCHÜRER, MD, PhD

Dr. A. Liyanage is with the Department of Community Medicine at University of Ruhuna in Galle, Sri Lanka. Dr. G. Liyanage is with the Department of Pharmacology at University of Ruhuna in Galle, Sri Lanka. Dr. Sirimanna is with National Hospital of Sri Lanka in Colombo Sri Lanka. Dr. Schürer is with the Department of Dermatology at the University of Osnabrueck in Osnabrueck, Germany.

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OBJECTIVE: Skin lightening agents are popular in southern Asia, but there is dearth of evidence on their effectiveness on Fitzpatrick IV/V skin types. This study was designed to assess the depigmenting efficacy of commercially available and specifically formulated ointments using the Mexameter® (MX 18). **METHODS:** This single center prospective study was performed to test five commercially available preparations (Eldopaque®, Aziderm®, Garnier Dark Spot Corrector®, Ban a Tan Cream® and Neostrata Pigment Lightening Gel) on 28 healthy female volunteers in Phase 1, while five single active ingredients in lipophilic dispersion (hydroquinone 4%, ascorbyl palmitate 1%, resveratrol 1% arbutin 5% and azelaic acid 20%) were tested on a different group of 26 healthy female volunteers in Phase 2. The test agents were applied twice a day for five days per week and continued for six weeks in both study phases. Weekly Mexameter® measurements were obtained from test sites and negative controls. **RESULTS:** Significant hypopigmentation when compared to untreated controls was observed with Aziderm cream ($p<0.05$, MWU) and the Neostrata Pigment Lightening Gel ($p<0.05$, MWU). All formulated preparations showed significant reduction in pigmentation; however, only the arbutin (5%) containing formulation revealed significant attenuation of pigmentation in comparison to the inactive control ($p<0.05$, MWU). **CONCLUSION:** All applications containing active ingredients showed significant skin lightening; however, only arbutin was able to demonstrate significant diminution of pigmentation when compared to the inactive control. **KEY WORDS:** Skin lightening preparations, Fitzpatrick IV/V skin type, Mexameter®

Acquired pigmentary disorders are common distressing dermatological problems among individuals with dark skin tones. They can occur secondary to skin diseases, systemic diseases, or exogenous causes. Melasma is the most common hyperpigmentary disorder among Asian people, particularly in females.[1] The cosmetic effect of pigmentary disorders can cause distress and poor quality of life.[2] Numerous prescription depigmenting agents exist to treat pigmentary disorders, as well as over the counter (OTC) formulations in the form of "fairness creams." These agents act on various steps of melanogenesis in order to reduce and/or inhibit pigmentation. However, the effectiveness and safety of these OTC formulations have not been adequately assessed. Commonly used depigmenting agents include hydroquinone, arbutin, azelaic acid, kojic acid, ascorbic acid and resveratrol.

Hydroquinone was considered to be the gold standard to treat acquired pigmentary disorders, particularly melasma. Oxidization of hydroquinone within melanocytes produces melanocytotoxic chemicals, including quinones, and are capable of suppressing the metabolic processes of the melanocyte.[3] These cytotoxic compounds are responsible for the destruction of pigment cells, resulting in skin depigmentation. In addition, hydroquinone acts on the rate limiting enzyme tyrosinase, thereby inhibiting the enzymatic oxidation of tyrosine to dopaquinone. It is common for hydroquinone to have adverse effects, particularly among individuals with dark skin tones, including skin irritation and allergic contact dermatitis. Rare instances of permanent depigmentation and exogenous ochronosis have been reported with long-term use.[4,5] Therefore, hydroquinone has been banned for use as skin whiteners in skincare cosmetics in Europe by the Cosmetic Directive 2000/6/EC. However, in the United States (US), hydroquinone is available for non-cosmetic use, both as prescription and OTC products in concentrations ranging from 0.4% to 5.0%. Since its medical use is under the purview of US Food and Drug Administration (FDA), hydroquinone-containing OTC products are no longer generally recognized as safe and effective.[6] In Sri Lanka, hydroquinone-containing products are currently available in combination preparations, both on prescription as well as OTC. However, the product Eldopaque cream®, which contains hydroquinone alone, has not been available since 2016 due to changes by drug importing agencies.

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CORRESPONDENCE: Achala Liyanage, MD; Email: achalaliyanage@yahoo.com

Arbutin, (hydroquinone-O-beta-D-glucopyranoside) is a naturally-occurring derivative of hydroquinone, used in its synthetic form to lighten skin. It acts by a tyrosinase-dependent pathway and its efficacy has been demonstrated.[7] The release of hydroquinone from arbutin is slow and dependent on glycosylases, as listed in Annexure III (entry 14) of the Cosmetics Directive 76/768/EEC. However, in 2015, the Scientific Committee on Consumer Safety (SCCS) considered the use in cosmetic products of α-Arbutin to be safe for consumers at concentrations of up to 2 percent in face creams and up to 0.5 percent in body lotions. The use of β-arbutin was considered to be safe in cosmetic products at concentrations up to 7% in face creams, provided that the contamination of hydroquinone in the cosmetic formulations remains below 1 ppm.[8] The sole *in-vivo* study performed with arbutin in the form of deoxyarbutin has shown overall skin lightening and improvement in solar lentigines after 12 weeks' application.[7]

Azelaic acid is a dicarboxylic acid derived from *Pityrosporum ovale*. It inhibits tyrosinase, mitochondrial oxidoreductase activation and DNA synthesis.[9] It has a significant effect on densely-pigmented melanocytes. Azelaic acid has been shown to be safe and effective in studies on acne-induced post inflammatory hyperpigmentation.[10] In addition, a non-randomized clinical trial in a Middle Eastern population has shown a superior effect of 20% azelaic acid compared to 4% hydroquinone in reducing mild melasma.[11] However, the efficacy of azelaic acid versus hydroquinone is controversial.[12, 13]

Topical stable vitamin C, magnesium L-ascorbic acid-2-phosphate (MAP), has also been shown to lighten pigmentation. It has been shown that ascorbic acid and related ingredients (magnesium ascorbyl phosphate, ascorbyl palmitate, ascorbyl dipalmitate, ascorbyl stearate, erythorbic acid, and sodium erythorbate) are safe for use as cosmetic ingredients.[14] Unfortunately, they rapidly lose their efficacy due to instability. Therefore, newer ascorbic acid derivatives with enhanced stability have been produced. An in vivo study using topical 10% magnesium l-ascorbic acid-2-phosphate showed a significant lightening effect on chloasma or senile freckles.[15] Additionally, ascorbic acid has been administered as a depigmenting agent to the skin via iontophoresis in some clinical trials, with variable results.[16, 17] However, safety concerns associated with contact dermatitis have been reported.[18]

Flavonoids (i.e. plant polyphenols) are used as depigmenting agents, alongside their widespread clinical uses as anti-inflammatory, anti-viral, anti-oxidant, and anti-carcinogenic mediators.[19, 20] Resveratrol (3,4,5-trihydroxystilbene) is a skin lightening flavonoid, acting by tyrosinase inhibition. Both *in-vitro* and *in-vivo* models have revealed inhibitory effects of melanin synthesis by resveratrol.[21] The compound reduces the expression of tyrosinase related proteins TRP-1 and/or TRP-2 and microphthalmia-associated transcription factor (MITF) in melanoma cells.[22,23] Another flavonoid used as a depigmenting agent is licorice. Licorice root extract (*Glycyrrhiza glabra, Glycyrrhiza uralensis*) is a common ingredient of skin-lightening products. The active compound of licorice root extract is glabridin, which acts by preventing UVB-induced pigmentation and inhibiting tyrosinase activity, superoxide anion production and cyclo-oxygenase activity.[24] In addition, the flavonoids in licorice act as competitive inhibitors of tyrosinase, as well as through other mechanisms. For example, liquiritin in licorice acts by dispersing the melanin and giving the effect of lightening the skin.[25] It has been shown that the depigmenting efficacy of licorice is greater than that of hydroquinone.[26] However, licorice in oil soluble extracts in whitening preparations may cause allergic contact dermatitis.[27]

Kojic acid is also widely used as a depigmenting agent. Kojic acid is derived from several species of fungi and acts as a tyrosinase inhibitor. The inhibitory effect of kojic acid on tyrosinase activity *in-vitro* is lower than that of arbutin at concentrations not affecting cell viability.[28] Further, *in-vitro* combinations of kojic acid with other whitening agents have shown potential synergistic effects.[29] A comparative study on the effectiveness of the skin lightening properties of hydroquinone and kojic acid revealed comparable efficacy.[30]

Topical niacinamide is believed to influence cutaneous pigmentation via a tyrosinase-independent pathway by down-regulating the transfer of melanosomes from melanocytes to keratinocytes and by interfering with the cell-signaling pathway between keratinocytes and melanocytes.[31] Bissett et al. demonstrated a 35 to 68-percent reduction in melanin transfer and significant improvement of cutaneous pigmentation. In addition, N-Nicotinoyl tyramine, a novel derivative of niacinamide made by conjugation with tyramine, has been shown to inhibit MITF and tyrosinase expression in murine melanoma cells.[32] Topical niacinamide has shown a significant, non-inferior lightening effect compared to hydroquinone in melasma patients.[33]

TABLE 1. Formulations of skin lightening preparations and negative control

FORMULATION	INGREDIENTS
1	Vegetable oil – negative control
2	Vegetable oil, 4% hydroquinone
3	Vegetable oil, 1% ascorbyl palmitate
4	Vegetable oil, 1% resveratrol
5	Vegetable oil, 5% arbutin
6	Vegetable oil, 20% azelaic acid

Today, a wide array of lightening agents is available; however, there is dearth of evidence on the effectiveness of depigmenting agents in Fitzpatrick IV/V skin types. The goal of the study was to compare available OTC products containing proven active ingredients available on the Sri Lankan market or the internet, and direct intra individual comparison of the efficacy of various depigmenting agents by using the Mexameter® (MX 18) on Sri Lankan Fitzpatrick type IV/V skin types.

METHODS

Study design and setting. This single center prospective study was conducted at the National Hospital of Sri Lanka, Colombo. This study was carried out in two phases: In Phase 1, five commercially available whitening products were tested to evaluate the efficacy of whitening. However, as the commercially available products differ in their galenic formulations, the second phase was conducted comparing five active ingredients, each in olus (vegetable) oil.

Phase 1. *Participants.* Each volunteer signed an informed consent form and was in good general health. All patients were from Sri Lanka and had Fitzpatrick IV/V skin types.

The sample size was calculated using a formula to detect significant difference between two proportions[34], based on the results of a previous study that assessed the

TABLE 2. *P*-values of differences in skin color between patients using lightening creams compared with the negative control during the study period (Phase 1)

LIGHTENING CREAM	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6
Eldopaque®	0.640	0.909	0.193	0.147	0.279	0.095
Aziderm®	0.026*	0.026*	0.022*	0.020*	0.101	0.208
Garnier Dark Spot Corrector®	0.512	0.600	0.533	0.441	0.279	0.350
Ban a Tan®	0.068	0.114	0.152	0.125	0.294	0.159
Neostrata Pigment Lightening Gel®	0.014*	0.016*	0.005*	0.008*	0.026*	0.043*

* *p*-value <0.05, Mann-Whitney test

total improvement in melasma when using 4% hydroquinone cream[35] Assuming a type I error of 0.05 and 80% power using a two-sided test, the minimum sample size required to detect a significant difference was 20. In Phase 1 of the study, healthy female volunteers from the hospital staff participated. Thirty-one female volunteers with no known skin diseases were selected for the study.

Treatment protocol. Three test areas, each 2.5×2.5 cm^2, were marked on the inner side of each upper arm, giving six test areas in total. The inner side of the upper arm was chosen; hence it was easily accessible and not commonly exposed to UV light. Skin color was objectively assessed by using the Mexameter®® (MX 18, Courage + Khazaka electronic GmbH, Cologne).

The Mexameter® is a tool used to assess both pigmentation (melanin content) and erythema (hemoglobin). The measurement is based on the absorption/reflection of specific wave-lengths, which are produced by the light emitting diodes of the probe. It is a convenient, non-invasive, reliable and widely-used research tool.[36, 37]

Five skin-lightening products available in Sri Lanka, containing proven active ingredients were selected and purchased over the counter or online, and compared with the negative control. These products included Eldopaque® cream, Aziderm®cream, Garnier Dark Spot Corrector®, Ban a Tan® cream, and Neostrata Pigment Lightening Gel®. The negative control site was not treated with any ointments, but was reviewed and measurements were taken using the Mexameter® along with the other application sites during the study period.

Eldopaque cream contain 4% hydroquinone and Aziderm contains 20% azelaic acid as the primary active ingredient. However, Garnier Dark Spot Corrector, Ban a Tan and Neostrata Pigment Lightening Gel have combinations of active skin lightening ingredients. Garnier Dark Spot Corrector contains ascorbic acid (pure vitamin C), retinol, *Gentiana lutea* root extract, geraniol, arbutin, limonene, linalool, salicylic acid, sodium methylparaben and tocopheryl acetate, all of which are known to have a skin lightening effect. Ban a Tan cream contains alpha arbutin 1% w/w, licorice extract 0.5% w/w, lumiskin and mulberry 1% w/w. Neostrata Pigment Lightening Gel contains 10% glycolic acid and a combination of kojic acid, citric acid, phytic acid, ferulic acid and bisabolol, which helps to reduce hyperpigmentation.

A defined amount of ointment (0.25 mL) was applied to each area using syringes. The frequency of application was twice a day, five days per week, for a total duration of six weeks. Considering the epidermal turnover time, the outcome objective assessment was set at six weeks.[38, 39] Daily review was performed visually by the principal investigator (dermatologist).

The intensity of the skin color of each area was determined at weekly intervals using the Mexameter®. The treatment areas were compared with baseline values and the negative control. Photodocumentation of both arms was employed prior to and at the end of the phase one study. Early discontinuation of individual local application during the study period was planned if local irritation, or intense depigmentation or hyperpigmentation developed at the site of application.

Phase 2. *Participants.* A new group of female volunteers from the hospital staff were enrolled. Twenty-eight female volunteers with no known skin diseases were selected for the study.

Treatment protocol. Skin lightening preparations, each containing one single active ingredient [hydroquinone (4%), ascorbyl palmitate (1%), resveratrol (1%), arbutin (5%) or azelaic acid (20%)] in identical formulations were applied using the same methodology as phase one of the study.

The active ingredients chosen reflected the above mentioned OTC products, except for kojic acid, which was replaced by resveratrol considering the safety assessment by Cosmetic Ingredient Review (CIR) Expert Panel.[40]

The test formulation (a lipophilic dispersion as opposed to an emulsion) employed an oil thickener (plant-based triglycerides). Each of the five preparations included one active ingredient, except for the negative control. Therefore, all preparations tested had the same formulation (Table 1).

As in the first phase of the study, formulations of five skin lightening preparations and the negative control were applied to a given test area twice daily, five days a week, for six weeks, on the inner side of each upper arm. The intensity of the skin color was determined at weekly intervals using the Mexameter®.

This study was approved by the institutional ethics committee of the National Hospital of Sri Lanka, Colombo, and was compliant with the principles of the Declaration of Helsinki.

Statistical analyses. The statistical significance of differences between given preparations and the negative control (Mann-Whitney U test) and/or between baseline and after 1–6-weeks of treatment (Wilcoxon test) was analyzed using SPSS® (Version 17; IBM Corporation, Somers, NY, USA). *P*-values <0.05 were considered significant.

RESULTS

In Phase 1, 28 of 31 volunteers completed the study. Volunteer age ranged from 22 to 57 years. The median (IQR) age was 42 (33.0–46.7) years.

When compared to the baseline, Eldopaque cream (4% hydroquinone) revealed significant hypopigmentation at the end of third week (-7, 96% Wilcoxon $p<0.05$). Garnier Dark Spot Corrector (5% ascorbic acid), reduced pigmentation was noted in comparison to the baseline after five weeks of continuous application (-2, 77% Wilcoxon $p<0.05$). The site where Ban a tan cream® was applied showed significantly less pigmentation at the second, third and sixth week of treatment (-3, 55% Wilcoxon $p<0.05$) compared to the baseline. However, compared to the untreated control, neither the Eldopaque cream, Garnier Dark Spot Corrector, nor Ban a Tan cream application sites showed significant diminution of pigmentation at all time points (Table 2).

Aziderm cream lightened the site of application compared to baseline at the end of fourth and sixth week (-5, 38% $p<0.05$

TABLE 3. *P*-values of differences in skin color between baseline and Weeks 1 to 6 (study Phase 2) in patients using lightening creams

TIME POINT	HYDROQUINONE 4%	AZELAIC ACID 20%	ASCORBYL PALMITATE 1%	ARBUTIN 5%	RESVERATROL 1%	CONTROL
Week 1	0.713	0.899	0.899	0.943	0.485	0.066
Week 2	0.545	0.232	0.554	0.134	0.509	0.611
Week 3	0.115	0.015*	0.019*	0.003*	0.025*	0.416
Week 4	0.022*	0.027*	0.011*	0.003*	0.011*	0.112
Week 5	0.071	0.005*	0.022*	0.005*	0.008*	0.424
Week 6	0.021*	0.001*	0.001*	0.001*	0.001*	0.107

*p-value <0.05, Mann-Whitney test

Wilcoxon). Furthermore, compared to the untreated control, Aziderm cream caused significant diminution of pigmentation from 1 to 4 weeks post application (Table 2). Photo documentation illustrated leftover cream particles at the application sites of Aziderm cream® (Figure.1).

Neostrata Pigment Lightening Gel demonstrated significant lightening at the end of the third week of treatment compared to baseline (-4, 24% $p<0.05$ Wilcoxon). Moreover, compared to the untreated control, hypopigmentation was recorded at all-time points ($p<0.05$, Mann-Whitney U test [MWU]). There was no local irritation or dyspigmentation noted at any site with any of the agents.

In Phase 2 of the study, 26 of 28 female volunteers completed the study period of six weeks. Age ranged from 22 to 57 years, with median (IQR) age being 40 (32.7–45.5) years.

Compared to baseline, the hydroquinone (4%) containing formulation showed significant lightening after the fourth and sixth weeks of application ($p<0.05$, Wilcoxon). Azelaic acid (20%), ascorbyl palmitate (1%) and resveratrol (1%) containing formulations revealed significant lightening at the end of the third week at given application sites (Wilcoxon, $p=0.015$, $p=0.019$, and $p=0.025$ respectively); however, when compared to the untreated control (MWU), none of the aforementioned agents showed significant pigmentary changes at all-time points. The arbutin (5%) containing formulation revealed significant attenuation of pigmentation by the end of week 3 ($p=0.003$, Wilcoxon) and remarkably, it remained significant in comparison to the inactive control ($p=0.0039$, MWU) throughout the study period. No considerable drop in pigmentation was observed in the inactive formulation throughout the study (Figure 2 and Table 3). Participants did not experience any local irritation or pigmentary change at any site with any of the formulations.

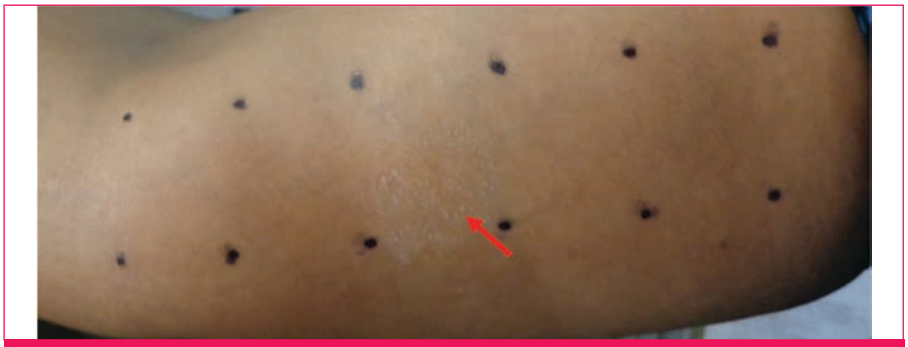

FIGURE 1. Persisting cream particles (red arrow) at the application site of the azelaic acid-containing commercial preparation (Phase 1).

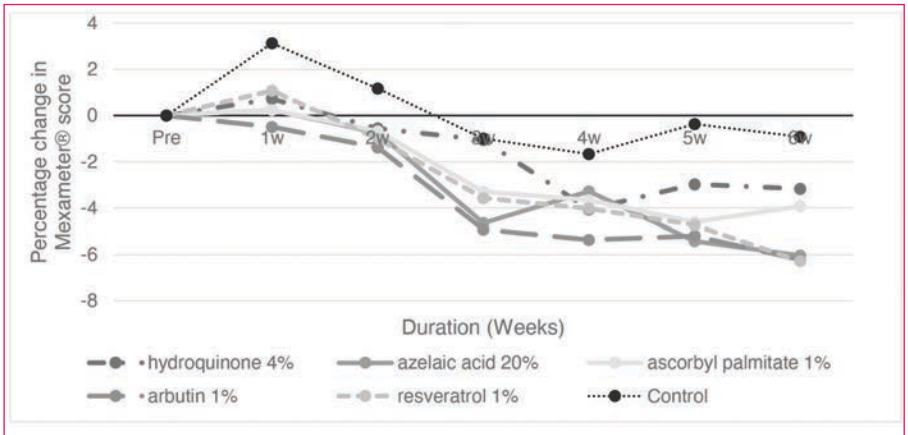

FIGURE 2. Percentage change in mean Mexameter® scores compared to pretreatment Mexameter® value at sites of application in the Phase 2 study. Different formulations, each containing single active ingredients, are represented by lines.

DISCUSSION

In Phase 1 of our study, all skin lightening products showed reduction of pigmentation compared to baseline. The products containing azeliac acid (Aziderm) and kojic acid (Neostrata Pigment Lightening Gel) were the only agents that showed statistically significant lightening compared to the untreated control. The kojic acid containing product (Neostrata Pigment Lightening Gel) was the most efficacious agent, with sustained significant results. These products contain a combination of lightening agents. For example, Ban a Tan cream contains not only arbutin, but also licorice extract, whereas Neostrata Pigment Lightening Gel contains kojic acid, lactic acid, licorice, gluconolactone, glycolic acid, citric acid, and ascorbic acid. Therefore, it is difficult to compare

the efficacy of over the counter products, as their formulations differ.

All applications containing active ingredients showed significant skin lightening by the end of the fourth week compared to baseline; yet only arbutin was able to demonstrate significant diminution of pigmentation when compared to the inactive control. Arbutin has been found to be the most effective active ingredient in our study, even more so than hydroquinone, despite being a naturally occurring derivative.

Both arbutin and hydroquinone suppress melanogenesis in the late stage of differentiation when the process becomes active. Thus, there is no significant difference in final melanin content between the compounds.[41] In addition, arbutin has been shown to have multiple effects on cells, besides the inhibitory effect on tyrosinase activity, such as antioxidant and anti-inflammatory effects.[42, 43] Furthermore, the stability of the lipophilic dispersions used for test formulations might have been more optimal for arbutin than for hydroquinone. This can be explained by the significant pigment lightening caused by arbutin. Choosing the best formulation for direct comparison of different active ingredients is a challenging task: considering their differences in lipophilicity and optimum pH.

In our study, since the test and control applications were applied to a single volunteer, it was possible to have age matched controls and eliminate intra individual variation. Furthermore, the topical ointments were applied to a non-sun exposed area to reduce the photo-induced pigmentary changes to the test area. However, the non-randomized study design, small sample size and relatively short follow up are limitations of this study.

CONCLUSION

All applications containing active ingredients showed significant skin lightening; however, only arbutin was able to demonstrate significant diminution of pigmentation when compared to the inactive control. Since studies regarding the effect of skin depigmenting agents in patients with Fitzpatrick type IV/V skin types are sparse, our findings on individual depigmenting agents will be useful for comparison with the results of future studies.

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