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Original Research Article

Efficacy and safety of topical trihydroxybenzoic acid glucoside and alpha-arbutin containing formulation along with a sunscreen in facial hyperpigmentation

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ABSTRACT

Background: Facial hyperpigmentation is a common presentation in Indian subjects and its treatment is complex. The aim of the study was to evaluate efficacy and safety of a 56-day skincare regimen of topical 3,4,5-trihydroxybenzoic acid glucoside (THBG/Brightenyl®) and α-arbutin containing formulation along with a sunscreen in the management of facial dark spots and melasma.
Methods: Thirty-six female subjects with facial dark spots or melasma who met inclusion/exclusion criteria were enrolled in a prospective, single-arm, study in India. Subjects applied a skin brightening cream containing 10% THBG and 2% α-arbutin twice daily in combination with a sunscreen (SPF=55) once daily; on their whole face for 56 days. Evaluation was carried out at baseline, days 28, 42 and 56 by the same dermatologist and technician. Efficacy was evaluated using mexameter, modified Melasma Area and Severity Index (mMASI), chromameter and cross-polarized light photography.
Results: There was a significant reduction in melanin content (mexametry) and mMASI score compared with the baseline. On chromametry, a significant improvement was seen in skin brightness/lightness (L*) and pigmentation [individual typology angle (ITA°)] demonstrating modification in skin color with an improvement in homogeneity/evenness of the skin tone (ΔE*) from the baseline. At day 56, the total area of the pigmentary spots on the cheeks showed a significant reduction compared to baseline using cross-polarized light photography. The treatment was well tolerated.
Conclusions: This study demonstrates that application of formulation containing 10% THBG and 2% α-arbutin along with a sunscreen is well-tolerated and efficacious in the management of facial hyperpigmentation like dark spots or melasma.

Keywords: Dark spots, Melanin, Melasma, Pigmentation, Skin brightening, THBG

INTRODUCTION

For centuries, diversity in human skin tone and appearance has been a subject of interest and research.[1] Skin pigmentation, one of the apparent variable phenotypes in humans, is regulated by melanocytes and keratinocytes.[2,3]

The Indian population shows a remarkable diversity in constitutive pigmentation with more than 2,000 ethnic groups. A majority of the population exhibit diversity in facial skin colour regardless of age and gender.[4] In India, pigmentation disorders cause psychosocial effects that can lead to repercussions on the quality of life.[1] A study in Northern India that assessed dermatoses in childhood and

adolescence demonstrated that 6.9% patients had pigmentary disorders. Pigmentary disorders were also reported in a study among 10.8% of adult patients attending dermatology clinic at a hospital in Western India.[5] The common presentations were hyperpigmentation, melasma, indistinct pigmented macules and periorbital hyperpigmentation. The most common clinical diagnosis of facial melanoses is Melasma (chloasma).[6] Women of reproductive age are generally affected by melasma as they are frequently exposed to ultraviolet (UV) radiation.[7-9] Hourbin et al reported in one of the first descriptive studies on Indian skin complexion and pigmentary disorders in 1204 women in four Indian cities that 20–30% of women aged 40–65 years present with facial melasma.[10] UV spots, recognized as one of the established measures of skin damage due to sun exposure, often result in non-visible mottled pigmentation leading to visible dark spots and uneven skin tone.[11,12] In a study with large sample size conducted in India, around 33% of middle aged women were affected by UV spots.[4,5,6,11] Management of melasma and similar pigmentary ailments is complex due to heterogenous nature of these conditions. However, protection from sunlight and depigmentation (skin lightening or brightening) is the first step towards a practical treatment approach. The current treatment option includes photoprotection by using sunscreen, topical compounds (like skin lightening/brightening agents), camouflage, bleaching agents, chemical peels, and laser and light therapies depending on skin type and condition.[13] To prevent the exacerbation of hyperpigmentation and to improve these conditions, photoprotection should be considered as a key adjuvant therapy.[14] Photoprotection can be done by the use of sunscreens, clothing and glasses.[15] Skin brightening/skin lightening/skin bleaching/depigmenting agents are widely considered a viable option to improve skin health, texture, tonality, complexion, and smoothness by reducing the melanin level.[16-19] Active molecules used in skincare formulations to modify skin color often include topical agents such as hydroquinone, arbutin, tretinoin, kojic acid, azelaic acid, vitamin C, fruit or plant extracts, licorice extract, and corticosteroids used alone or in combination with other agents.[20,21] Safety concerns have been identified with usage of hydroquinone, kojic acid, tretinoin, and corticosteroids.[13,22-24] In terms of safety, hydroquinone causes irritation and exogenous ochronosis. Regulatory agencies across the world have raised concerns over its safety and long term usage.[22] Kojic acid may undergo photo degradation making it difficult to obtain stable and effective product.[23] It may cause irritation, contact dermatitis and erythema.[14] Tretinoin takes a much longer time to act and causes side effects like erythema, burning, stinging, dryness, and scaling.[14] Long term use of corticosteroids on face can cause skin atrophy, telangiectasias, and/or an acneiform eruption.[13] Therefore, there is a necessity for effective topical depigmenting agents with fewer adverse effects suited for the Indian skin type.[20] An alpha-glucoside derivative of trihydroxybenzoic acid [trihydroxy benzoic acid glucoside (THBG); also known as Brightenyl®], is a stabilized derivative of THBA (3,4,5-trihydroxybenzoic acid), called 3,4,5-trihydroxybenzoic acid glucoside that was developed by the addition of the alpha-d-glucoside moieties to THBA. THBG is converted into THBA by skin microbiota expressing alpha glucosidase, an enzyme activity.[26] THBA and THBG when delivered in situ, have been shown to act in synergy on 7 biological targets that help regulate and optimize the skin complexion (Figure 1 and Table 1 describes the 7 mechanisms of action).[27] THBG 2% has previously demonstrated UV ray protectant effect and skin color modulation effect amongst Asian women.[21] THBG at 10% is found to be non-irritant and non-sensitizing as confirmed by 48 hour human patch test and human repeat insult patch test (HRIPT) respectively.[28] Alpha-arbutin significantly inhibits tyrosinase enzyme and melanin production and thus delivers a sustained improvement in skin lightening.[29,30] Alpha-arbutin inhibited the tyrosinase enzyme 10 times as strongly as β-arbutin.[31] Flashwhite Unispheres® has a high titanium dioxide concentration that brightens the complexion and homogenizes skin tone while blurring spots and other imperfections.[32] Unitamuron® H-22 has shown to improve skin hydration, smoothing effect and skin elasticity.[33] A novel formulation (Bristaa™ Intense – Sun Pharma) containing 10% THBG, 2% α-arbutin, 1% Flashwhite Unispheres® and 1% Unitamuron® H22 as active ingredients is developed in India for the management of dark spots and melasma. There exists the need to examine the beneficial effects of this formulation in the management of pigmentary disorders in Indian setting. Thus, our current study evaluated the efficacy and safety of the skincare regimen of combination of skin brightening cream containing 10% THBG and 2% α-arbutin along with a sunscreen to reduce dark spots and melasma.

Table 1: Effects of THBG on the skin.

S. no.	Mechanisms of action	Effect
1	It inhibits ROS production	Anti-oxidant
2	It prevents UV-induced DNA damages	Photoprotectant
3	It controls the expression of MITF	Stops the melanogenesis process
4	It reduces the expression of PGE2	Decreases vasodilation and redness
5	It controls the Nf-κB pathway	Reduces inflammation
6	It saturates keratinocytes receptors	Stops melanin transfer
7	It blocks melanin synthesis even under UV conditions	Brightens the skin

DNA=deoxyribonucleic acid; MITF=melanocyte inducing transcription factor; NF-κB=nuclear factor kappa-light-chain-enhancer of activated B cells; ROS=reactive oxygen species; PGE2=prostaglandin E2; THBG=trihydroxybenzoic acid α-glucoside; UV=ultraviolet

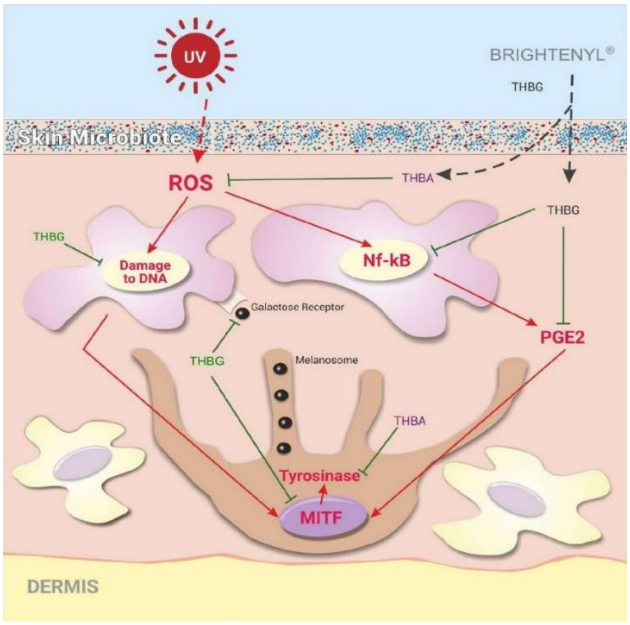

Figure 1: Mechanisms of action of THBG.

DNA: deoxyribonucleic acid; MITF: melanocyte inducing transcription factor; NF-κb: nuclear factor kappa-light-chain-enhancer of activated B cells; ROS: reactive oxygen species; PGE2: Prostaglandin E2; THBA: trihydroxybenzoic acid; THBG: trihydroxybenzoic acid α-glucoside; UV: ultraviolet

METHODS

Study design

This study was a prospective, single-arm study conducted at one study site (Mascot-Spincontrol India Private Limited) from December 2019 to February 2020.

A sample size of 36 female participants was deemed appropriate for the study. The treatment duration was 56 days and consisted of four study visits (±1 day) post-screening: baseline, day 28, day 42, and day 56 (Figure 2).

Study participants

Healthy Indian female subjects aged 18-45 years and presenting with visible facial dark spots or melasma (with at least one dark spot ≥3.5 mm in diameter) were included in the study if they had Fitzpatrick skin types III-IV; provided written informed consent; had healthy skin on the studied anatomic unit (free of eczema, wounds, and inflammatory scar); and did not have any signs of infectious and evolutive pathology. The subjects were excluded from the study if they had chronic dermatosis or cutaneous hypersensitivity; were smokers; were on any medications or procedures that could affect the pigmentation; were pregnant or breastfeeding or refused to follow the instructions during the study. Detailed exclusion criteria are described in supplementary Table 1.

Study treatment and administration

The skincare regimen used in the study, Bristaa™ Intense, a skin brightening cream and Photostable® Gold matte finish, a sunscreen gel with sun protection factor (SPF) of 55 were provided by Sun Pharma, Mumbai, India.

Bristaa™ Intense was applied twice daily (morning and evening) on the whole face. After applying the brightening cream, the sunscreen gel was applied once daily in the morning on the whole face. This daily regimen was followed for the 56-day study period. The subjects were blinded to the study treatment. Blinding was ensured by re-labeling of the study treatment products.

The application of local (topical)/systemic products or surgical/interventional techniques for a lightening/ brightening effect or a depigmenting effect was prohibited during the study period. Subjects were also instructed not to use the skincare regimen on the morning of the study visit (i.e., on the day of assessment).

Study endpoints

The study endpoints for efficacy in facial dark spots or melasma included evaluation of melanin reduction using mexameter; reduction in severity of melasma using modified Melasma Area and Severity Index (mMASI); improvement in the brightness/lightness of the skin color (L*), improvement in the eveness of the skin tone and skin homogeneity (ΔE*) and reduction in intensity of the skin pigmentation (ITA°) using chromameter and reduction in the visibility of pigmentary spots analyzed through its morphology (number and total occupied area) and its color difference with the normal skin color (ΔE*ab) using cross-polarized light photography.

The study endpoint for safety included an evaluation by the dermatologist.

Study assessments and measurements

A detailed history of the subjects, including medical history, personal habits, including possible allergies, skincare and make-up habits, were obtained before the study. For all subjects, mexametry, mMASI score, chromametry, and dermatologist evaluation were carried out at baseline, day 28, day 42, and day 56. For cross-polarized photography, images were taken at baseline and day 56 only. A 20-minute period of acclimatization was provided to each subject in an air-conditioned room (temperature: 20°C-25°C, relative humidity: 50±10%) before study procedures and assessments were carried out under the same conditions.

The same dermatologist and technician performed all the evaluations.

Mexametry: melanin content

The Mexameter® MX 18 (Courage and Khazaka Electronic, Cologne, Germany) is a narrow-band reflectance skin spectrophotometer that measures the melanin content of the skin.

At each study visit, the measurements were performed on the selected pigmentary spots (darks spots or melasma) for each subject. Three repeat measures were carried out on the selected pigmentary area. The average of the readings was considered, and results were expressed in arbitrary units.

mMASI: severity of melasma

Clinical assessment of quantification of melasma severity was recorded by mMASI score at each study visit, in subjects with visible facial melasma. The mMASI score was calculated by dividing the face into four specific areas: forehead (30% of the facial area), right malar region (right cheek and adjoining area of the nose: 30% of the facial area), left malar region (left cheek and adjoining area of the nose: 30% of the facial area), and chin (10% of the facial area) (supplementary Figure 1A). Area of involvement (A) in each of the four areas was rated from 0-6 with each number indicating the extent of involvement (0=absent/no involvement; 1≤10% involvement; 2=10-29% involvement; 3=30-49% involvement; 4=50-69% involvement; 5=70-89% involvement; and 6=90-100% involvement). Darkness (D) indicated melasma induced facial skin color change in each of the four areas and was rated from 0-4 with each number indicating the extent of hyperpigmentation compared to the normal skin color (0=absent/normal skin color; 1=slight; 2=mild; 3=moderate and 4=severe).

The mMASI score was calculated using the formula where A is the area of involvement and D is darkness.[34]

$$[0.3\ (A)(D)]_{forehead} + [0.3\ (A)(D)]_{left\ malar} + [0.3\ (A)(D)]_{right\ malar} + [0.1\ (A)(D)]_{Chin}$$

The total scores ranged from 0-24, with 0 being the least severe. A single dermatologist assessed the mMASI score throughout the study, to prevent calculation bias.

Chromametry: L*, a*, b*, ΔE* and ITA° parameters

Chromameter is a reflectance skin spectrophotometer that measures reflected light in the visible spectrum (range: 400-700 nm). The skin color and tone was measured using a Chromameter (CR-400 Konica Minolta, Japan). The skin parameters measured and/or calculated included: L*, a* and b*[26], ΔE* and individual typology angle (ITA°). L* describes relative brightness or lightness (and at opposite, darkness); a* describes a color ranging from green (negative value) to red (positive value); b* describes a color ranging from blue (negative value) to yellow (positive value); ΔE* describes evenness of the skin tone (skin homogeneity) and ITA° qualifies the skin tones from dark to fair. Table 2 provides a summary of these different parameters and their interpretations. The face was divided into six areas: forehead, left cheekbone, right cheekbone, chin, left cheek adjoining the nostril/mouth area and right cheek adjoining the nostril/mouth area (supplementary Figure 1B). Three repeat measures of L*, a*, b* of the selected location in each of the six areas were taken in each subject at all time points. ΔE* was calculated as per the readings for all the six facial areas. ITA° was calculated as per the readings for the two cheekbone's area. Only the average value is taken into account. The results are expressed in arbitrary units.

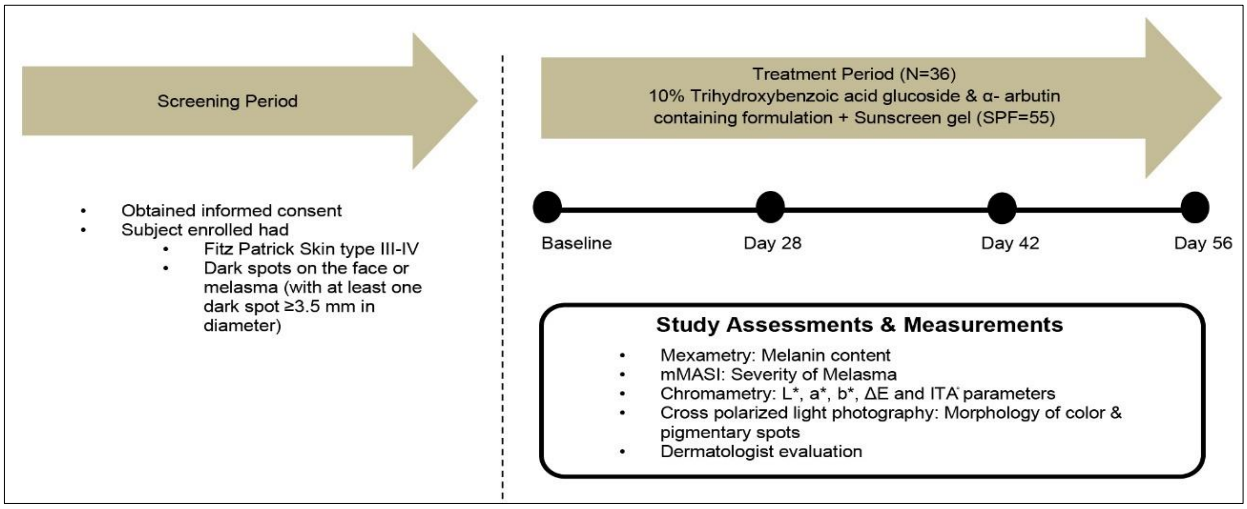

Figure 2: Study design.

a*: a color ranging from green (negative value) to red (positive value); b*: a color ranging from blue (negative value) to yellow (positive value); ΔE: evenness of the skin tone and skin homogeneity; ITA°: reduction in intensity of the skin pigmentation; L*: relative brightness or lightness (and at opposite, darkness); mMASI: modified melasma area and severity index; N: number of subjects; SPF: sunscreen protection factor

Table 2: Chromameter parameters for color assessment.[21]

Parameters	Determination methodology	Meaning	Value and interpretation
L*	Direct reading	Brightness of the color	From 0 to 100; L*=0: total black; L*=100: total white (maximum lightness)
a*	Direct reading	Chroma of the color	From -128 to +127 corresponding to 256 levels; -a*=green, +a*=red
b*	Direct reading	Chroma of the color	From -128 to +127 corresponding to 256 levels; - b*=blue, + b*=yellow
ΔE*	Equals to $[(L^*si–L^* sj)^2 + (a^*si – a^*sj)^2 + (b^*si–b^*sj)^2]^{1/2}$ (si and sj means two different sites defined on the face	Evenness of skin tone (skin homogeneity)	From 0 to 100; 0=less color difference, 100=complete color distortion
ITA°	Equals to arctan [((L*50)/b*)*(180/π)]	Intensity of pigmentation	Dark skin types: lower ITA°; light skin types: higher ITA°; if the ITA° increases, the quantity of melanin decreases

Individual typological angle (ITA°)

Cross-polarized light photography: morphology and color of pigmentary spots

Cross-polarized light photography enables better visualization of pigment changes in the skin. Pictures of the whole or three-fourth of the face were taken with a high-resolution camera using Nikkor 60 mm lens equipped with two filters (lens filter set perpendicular to the flash filter) to produce cross-polarized light. Photographs were taken at baseline and at day 56.

The visibility of pigmentary spots was assessed by analyzing its morphology and color.

Morphology

Number of pigmentary spots and total area occupied by the pigmentary spots (pixels²) on each cheek.

*Color (*ΔE*ab)*

The color difference between the single pigmentary spot selected on each cheek and the normal skin on a scale of 0-100, where 0 is less color difference, and 100 indicates a complete color distortion.

Dermatologist evaluation

The dermatologist evaluated the whole face of the subjects at each study visit for signs of erythema, edema, dryness, scaling, peeling, itching, and tingling using the following scale: none (0), slight (1), moderate (2), and severe (3).

Statistical analysis

The sample size calculated for this study was 36 (18 subjects each with dark facial spots and facial melasma).

The study variables were summarized using descriptive statistics, and the normality test was performed using the Shapiro–Wilk test with a 1% threshold. Continuous data were provided as mean±standard deviation (SD) and compared with the Wilcoxon test and student t-test. The significance threshold was fixed at 5%. Categorical variables were expressed as numbers and percentages from the baseline (except for the dermatological evaluation for efficacy). For dermatological grading, numbers and percentages of subjects presenting an improvement were recorded. The statistical difference in frequencies (%) between favorable and unfavorable opinions were evaluated using the Chi-squared test at 5%. All analyses were performed using SigmaStat v3.1 and Paleontological statistics software (PAST) v1.37.

Ethical considerations

This study was carried out in conformity with the principles outlined in the declaration of Helsinki, and all data, results, and subject anonymity were handled as confidential. The study was reviewed and approved by an independent ethics committee for the participating center. Written informed consent of all subjects was obtained before study participation, including their approval on the usage of their photographs and images for publishing purposes or duplication without direct identification.

RESULTS

Demographics and baseline characteristics

A total of 36 female subjects were enrolled in the study. One subject with melasma dropped out of the study after the baseline visit and was excluded from the data analysis. The mean age (±SD) of 35 subjects was 34.4 (±8.5) years. Thirty-four subjects completed all the study assessments. One subject with melasma could not be assessed at the last study visit due to an adverse event. Overall, no protocol deviations were observed during the study.

Efficacy assessments

Mexametry assessment

Post-treatment, the mean±SD melanin content of the pigmentary spot was 412.74±88.62 at day 28,

399.37±90.38 at day 42, and 391.06±100.91 at day 56, compared with 437.3±90.81 at baseline. The percent reduction in melanin content over 56 days was significant compared with baseline (p<0.001 each) as shown in Figure 3.

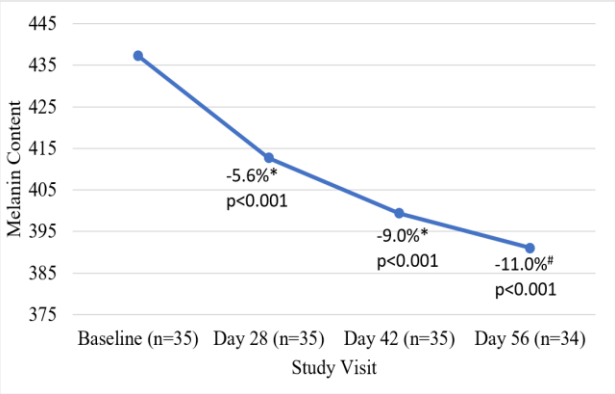

Figure 3: Reduction in melanin content (mexametry).
Change from baseline is shown in %; *Wilcoxon; #student t-test (two-tailed); p value versus baseline

mMASI score evaluation

The mean mMASI score in the melasma subjects at baseline, day 28, day 42 and day 56 are shown in Table 3. The change observed in the mMASI score was significant (p<0.001) throughout the study (Table 3).

Chromametry assessment

The findings of the chromameter analysis are summarized in Table 4. The study treatment demonstrated a statistically significant improvement on skin parameters of L* and ITA* with non-significant effects observed on the skin parameter of ΔE*. The L* parameter increased significantly after the treatment from day 28 (+0.42%) to day 56 (+1.12%) demonstrating a brightening effect on the skin. The effect seen was time dependent. The improvement in ITA° after treatment was significant from day 28 (+4.76%) to day 56 (+15.4%) in a time-dependent manner, corresponding to a skin-brightening effect. Numerical improvement was observed in the ΔE* parameter, i.e., skin tone evenness on the whole face at day 28 (3.41±0.73), day 42 (3.36±0.77) and day 56 (3.24±0.75) compared with baseline (3.40±0.83).

Cross-polarized light photography

At day 56, the total area of the pigmentary spots on the cheeks showed a significant 21.1% decrease (p=0.002, Wilcoxon test) to a mean±SD of 10612.74±10370.94 pixels^2 compared to 13455.06±10384.9 pixels^2 at baseline using cross-polarized light photography.

On the other hand, there was no significant reduction in the number of pigmentary spots or change in color (ΔE*ab) of the studied pigmentary spots on the cheeks after 56 days of treatment. Figure 4 is an illustration of the results obtained on representative subjects with dark spot and melasma.

Table 3: Modified melasma area and severity index (mMASI).

Study visit	N	mMASI score (mean±SD)	ΔmMASI		P value
			Absolute	Percentage	
Baseline	17	3.34±1.19	-	-	-
Day 28	17	2.70±1.16	0.64	19.2	<0.004*
Day 42	17	2.21±1.26	1.13	33.8	<0.001#
Day 56	16	1.70±1.00	1.54	46.1	<0.001#

*Wilcoxon's test; # student's t-test (two-tailed); ΔMASI=MASI score reductions after treatment; N=total number of subjects at each timepoint; SD=standard deviation

Table 4: Color parameters recorded (L*, a*, b*) or calculated (ITA°) on the cheekbones from a chromameter.

Study visit	Color parameters (mean ± standard deviation)					Average variation parameter vs baseline (%); p value	
	N	L*	a*	b*	ITA°	L*	ITA°
Baseline	35	54.04±2.71	12.38±1.22	19.77±1.17	11.36±7.64	-	-
Day 28	35	54.27±2.58	12.34±1.20	19.97±1.16	11.90±7.17	0.42%; p<0.008#	4.76%; p<0.024#
Day 42	35	54.35±2.56	12.68±1.32	19.73±1.15	12.31±7.17	0.58%; p<0.001*	8.32%; p<0.002*
Day 56	34	54.61±2.5	12.64±1.28	19.65±1.08	13.04±6.91	1.12%; p<0.001*	15.40%; p<0.001*

a*: a color ranging from green (negative value) to red (positive value); b*: a color ranging from blue (negative value) to yellow (positive value); ITA°: reduction in intensity of the skin pigmentation; L*: relative brightness or lightness (and at opposite, darkness); mMASI: modified melasma area and severity index; N: total number of subjects at each timepoint; significance was calculated versus baseline at each time point using *Wilcoxon test and #student t-test (two-tailed); statistically significant data is shown in bold

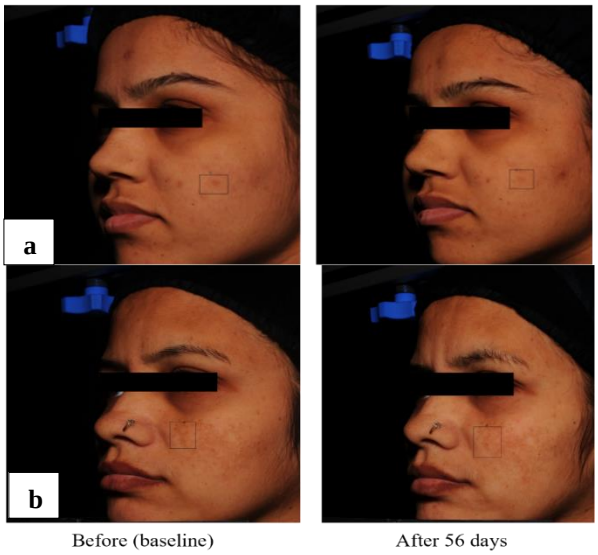

Figure 4: Cross-polarized photographs taken before and after THBG cream application for 56 days from (a) subject with dark spot; and (b) subject with melasma.

THBG: trihydroxybenzoic acid α-glucoside

Safety assessments

Dermatologist evaluation

On day 28, grade 1 (mild) dryness was noted in three subjects after treatment, and it resolved by end of the study. Throughout the study, no other clinical signs of erythema, edema, scaling, peeling, itching, and tingling were noted.

One subject with melasma reported redness on the cheeks and forehead (possibly related to the product) at the last study visit (day 56) and shared a history of significant sun-exposure for 2-days prior to the study visit. The redness was treated and it resolved completely within 3 days.

DISCUSSION

Most of the studies on skin brightening agents focus on modification of skin pigmentation based either on well-recognized objective tools like mexametry, chromametry, self-assessment and/or assessment by a trained technician using grading scales.[21] The current study evaluated the efficacy and safety of a skincare regimen (skin brightening cream containing 10% THBG and 2% α-arbutin along with a sunscreen) in Indian subjects with dark spots or melasma having Fitzpatrick skin type III-IV. The expected outcome was to reduce facial hyperpigmentation and improve overall lightening of the skin. Mexametry showed a significant decrease in the melanin content of pigmentary spots (darks spots or melasma), mMASI score showed a significant improvement in the severity of melasma and chromametry showed a substantial improvement in skin color parameters. In addition, cross-polarized light photography of pigmentary spots showed a significant reduction in the total area occupied by pigmentary spots. The product was well tolerated and no specific safety concerns were identified.

Chajra et al conducted a study with 2% THBG in 20 Asian subjects with clinical signs of hyperpigmentation (face spots) and redness.[12] The subjects applied either a vehicle cream on one side of their face or a cream containing 2% THBG, twice a day for 84 days. The parameters studied were L* and ITA° (chromametry) and skin melanin content SIAscope™. The results showed improvement in L* by 1.8% (p=0.001) on day 56 and 2.2% (p=0.0039) on day 84, when compared to the baseline. Also, an improvement by 10.4% (p=0.0008) on day 56 and 11.5% (p=0.0034) on day 84 in ITA° was seen, compared to the baseline. THBG cream induced a significant decrease of skin melanin content from 5.1% (p=0.0174) to 7.5% (p=0.0017) from 56 to 84 days of daily use. In *in vitro* studies using the 2,2-diphenyl-1-picrrylhydrazyl (DPPH) reduction test, the biological efficacy of THBA in synergy with THBG was shown to be 4 times more compared to vitamin C in capturing reactive oxygen species (ROS) thereby reducing skin damage.

Kojic acid and its combinations is one of the skin brightening agent used in India. Desai et al studied multi-ingredients facial serum containing 1% kojic acid in 55 subjects having Fitzpatrick skin types I-IV with mild to moderate hyperpigmentation.[34] The facial serum was applied twice daily along with a sunscreen of SPF 70 for the period of 12 weeks. The study evaluation parameters included mMASI and mexametry only. A significant reduction in melasma, as evaluated by the mMASI score (-0.98; p<0.001) was seen at the end of 12 weeks of treatment. On week 12, the decrease in melanin index (MI) was significantly higher for the lesional melasma skin as compared to the control site (p=0.028).

Facial hyperpigmentation is a major dermatological challenge, with a high prevalence in Indian population. Management of facial hyperpigmentation still remains complex and challenging for the dermatologists. The available treatment options have safety concerns and are therefore used for short duration. Our study with 10% THBG and 2% α-arbutin containing formulation along with a sunscreen proved to be an efficacious and safe treatment for the subjects with facial hyperpigmentation.

While this is the first study of formulation containing 10% THBG with 2% α-arbutin in India, limitation of its short duration should be acknowledged as any treatment for facial hyperpigmentation is recommended for 3 months or longer.[35] However, a key strength of our study was the use of objective quantitative and qualitative tools like mexameter, mMASI, chromameter and cross-polarized light photography.

A study with similar design as the current study, enrolling 124 Indian subjects with melasma or dark spots with same

skincare regimen applied for the duration of 90 days is ongoing in India (CTRI/2021/11/038345).

CONCLUSION

The results described herein demonstrated that treatment with skincare regimen (Skin brightening cream containing 10% THBG and 2% α-arbutin along with a sunscreen) provided a statistically significant reduction of studied parameters in dark spots and melasma over 56 days. The lack of adverse effects further encourages using this product to treat facial hyperpigmentation and is expected to promote greater acceptability amongst Indian subjects.

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Conflict of interest: Dr. Mukesh Gabhane, Dr. Poonam Rohira, Dr. Priyank Shah are full time employees of Sun Pharma. Dr. Siddheshwar Mathpati has received investigator fee for study conduct through the Contract Research Organization - Mascot Spincontrol India Private Limited
Ethical approval: The study was reviewed and approved by an Independent Ethics Committee for the participating center

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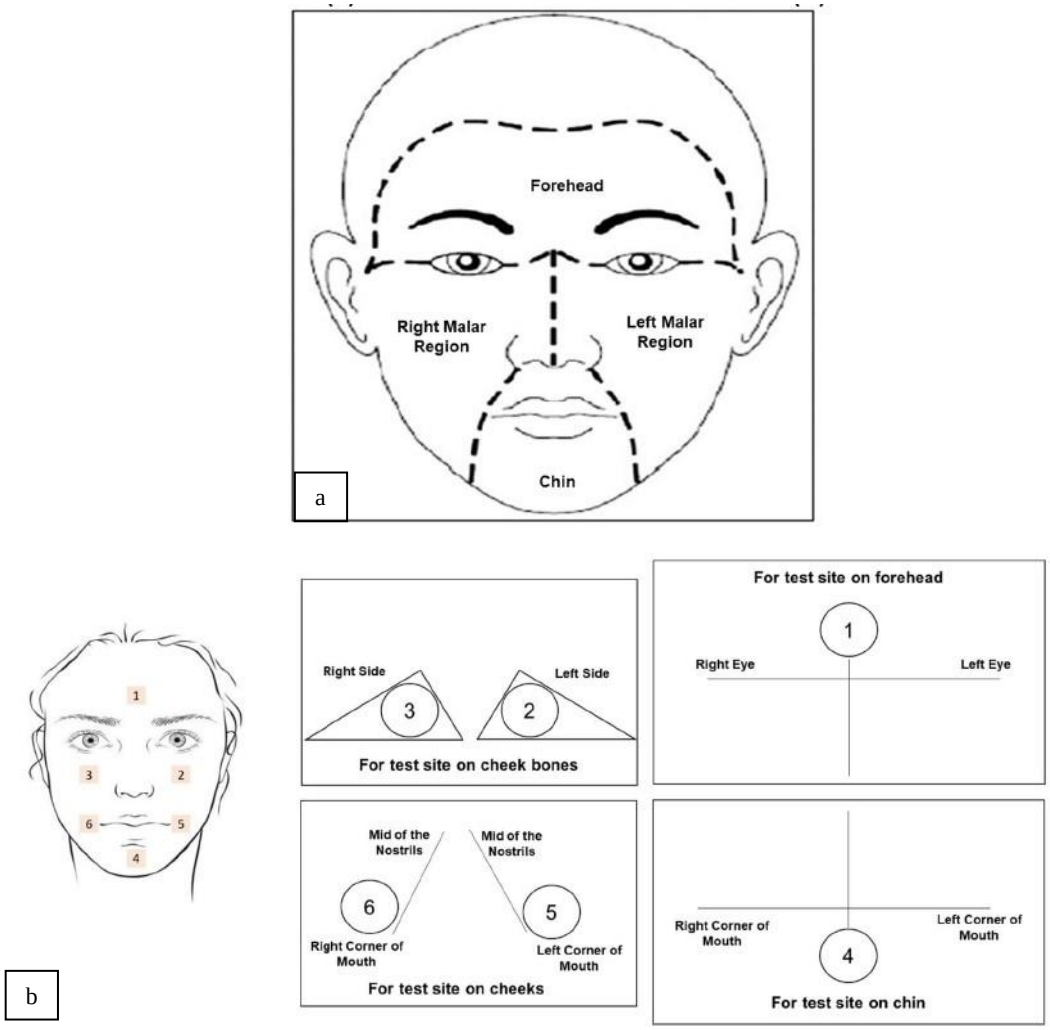
SUPPLEMENTARY

Supplementary Table 1: exclusion criteria.

Standard criteria	• Chronic dermatosis liable to modify the cutaneous reactivity on the tested area • Insulin-dependent diabetic or non-insulin-dependent diabetic with a recent therapy (<6 months) • Progressive asthma (either under treatment or last fit in the previous two years) • Epilepsy • Non-stabilized thyroid problems (requirement of a stabilized treatment for at least six months) • Cutaneous hypersensitivity • Pregnant or breastfeeding or having stopped to breastfeed in the past three months • Refused to sign the consent form for study participation • Taking part in another study liable to interfere with the current study • Diagnosed or highly probable allergy to one or several compounds of the cosmetic products or food products or latex • Following a chronic medicinal treatment comprising any of the following products: aspirin-based products, anti-inflammatories, antihistamines, corticotherapy • Undergo a surgery requiring a general anesthetic of more than one hour in the past six months. • Change in the cosmetic habits in the 14 days preceding the start of the study on the studied anatomic unit • Applied cosmetic product (including make-up) on the studied areas the first day of the study
Specific criteria	• Started, changed, or stopped a hormonal treatment (hormonal contraception, Hormone Replacement Therapy) in the past three months. • Started, changed, or stopped her tobacco consumption (for smokers consuming more than ten cigarettes per day) in the previous six months • Used a medicinal treatment that could lead to hyperpigmentation (phenytoin, amiodarone, metals, minocycline) in the previous six months • Took oral supplements with major or minor effects on whitening of skin (e.g., vitamin C, beta-carotene) • Had beauty treatment (e.g., skin cleansing, exfoliation, scrub, mask) or having applied self-tanning products in the week preceding the start of the study • Applied products with anti-wrinkle action (Retinoic acid, retinol, retinaldehyde, isotretinoin) in the two weeks before the start of the study • Applied products with a depigmenting action (hydroquinone or derivates) in the four weeks preceding the start of the study. • Undergo physical and/or chemical treatments of the spots (liquid nitrogen, dry ice, pulsed flash lamp, dermabrasion, chemical peel) in the previous six months • Suntanned skin on the studied areas that could interfere with the study evaluations
Refusing to follow the instructions during the study	• Not taking part in any family planning activities leading to pregnancy and breastfeeding • Not taking part in another study liable to interfere with the current study • Not taking medicinal treatment comprising the following products: aspirin-based products, anti-inflammatories, antihistamines, corticotherapy, taken by general or local routes (the only medication permitted is paracetamol) • Not changing their cosmetic habits apart from the conditions mentioned in the protocol, on the studied anatomic unit • During the study: Do not use other cosmetic products than the tested products to the studied areas (only make-up for the lips and eyes are accepted) • On the day of the measurements: No test product must be used (only face cleaned with water is accepted) • Not starting, changing, or stopping a hormonal treatment (hormonal contraception, Hormone Replacement Therapy) • Not beginning a medicinal treatment which could lead to hyperpigmentation • Not taking oral supplements with major or minor effects on whitening of skin (e.g., vitamin C, beta-carotene) • Not starting an oral or local retinoid-based treatment • Not taking beauty treatment (e.g., skin cleansing, exfoliation, scrub, mask) or applying self-tanning products • Not using products or techniques or surgery with a depigmenting action • Not practicing water activities (swimming pool, sauna, hammam, balneotherapy) • Not undertaking sports on the days of study • Not exposing oneself to the sun by respecting a strict photo-protection



Supplementary Figure 1: (a) mMASI score (a) mMASI score: area of involvement (A), (b) sites for chromametry assessment.