Evaluation of the whitening properties of combined kojic acid, arbutin, sepiwhite® and achromaxyl® vs. 2% and 4% hydroquinone in the treatment of melasma

Estudo clínico para a avaliação das propriedades clareadoras da associação de ácido kójico, arbutin, sepiwhite® e achromaxyl® na abordagem do melasma, comparada à hidroquinona 2% e 4%

ABSTRACT

Introduction: Melasma is a common, acquired, long-lasting skin disorder that is often resistant to treatment and causes negative psychological effects on patients.

Objectives: To evaluate the efficacy, safety, and tolerability of the topical combination of kojic acid, arbutin, sepiwhite® and achromaxyl® compared to 2% and 4% hydroquinone in the treatment of facial melasma.

Methods: A single-blind, comparative, monocentric clinical study with 120 volunteers (aged 18-50, I to IV Fitzpatrick skin types) was conducted. The study population was divided into 3 groups: Group A (n = 40; Blancy®, 2 times a day), Group B (n = 40, 2% hydroquinone at night), and Group C (n = 40, 4% hydroquinone at night), and instructed to use the study product for 90 consecutive days. Clinical (classification and quantification of melasma) and photographic evaluations were carried out, and a questionnaire assessed the impact on the patients’ quality of life and the products' general efficacy.

Results: Most volunteers (n = 102, 85%) completed the study (Group A = 34, Group B = 33, Group C = 35). The Melasma Area Severity Index metrics presented a statistically significant decrease (i.e., improvement) throughout the study in all three groups (p-value < 0.001).

Conclusion: The topical use of the kojic acid, arbutin, sepiwhite® and achromaxyl® combination proved to be an effective and safe alternative for treating melasma.

Keywords: melanosis; hydroquinones; arbutin; quality of life.
INTRODUCTION

Physical appearance is important in today’s society; cutaneous patches especially on the face, can cause psychosocial disorders due to their unattractive nature. Approximately 10% of the population has some facial imperfection – such as scarring, stains or deformities – that affects the affected individual’s daily routine.

The constant search for an improved physical appearance is a sociocultural phenomenon, and very frequently ranks higher than one’s professional, economic, or emotional satisfaction. Pigmentary skin alterations such as melasma, postinflammatory hyperpigmentation, and drug-induced hyperpigmentation have a prolonged course and are often resistant to treatment, which contributes to the negative psychological impact of affected individuals.

Melasma is a common acquired skin disorder characterized by brown-grayish or dark brown stains, which are symmetrical and have irregular borders, and often affects the face of women of childbearing age. It predominates in young female patients with higher phototypes (of Hispanic, African, or Asian descent), however it can affect both genders and can begin after menopause. Its denomination derives from the Greek word melas, which means black.

Melanocytes, where the genesis of melasma takes place, are dendritic cells responsible for skin and hair pigmentation, and derive from melanoblasts that are originally from neural crest cells. During embryogenesis, they migrate through the mesenchyme to the epidermis and hair follicles, and are also found in the leptomeninges, cochlea, and uveal tract (ciliary body, choroid, and iris).

Melanin, a polymer of high molecular weight that contains nitrogen, assumes a dark brown color, and is considered the main pigment that determines the color of the skin – thus it also participates in the filtration and absorption of UV rays. The enzyme responsible for the production of melanin is tyrosinase, which participates in the hydroxylation of tyrosine and the oxidation of DOPA.

The melanocytes located in the epidermal basal layer protrude their dendrites through the stratum spinosum, transferring their melanosomes (also the location of melanin biosynthesis, known as melanogenesis) to keratinocytes. This set then forms the epidermal-melanin unit, which comprises one melanocyte and 36 keratinocytes. This unit can sometimes be found in the dermis.

Epidermal hyperpigmentation – both primary (constitutional) and secondary (caused by drugs or melanocytic hyperreactivity) – is therefore due to the excess production of melanin. Melanin production is influenced by several factors, such as solar radiation, melanocyte stimulating hormone (MSH), endothelin-1, basal fibroblast growth factor, enzymes and tyrosinase-stabilizing protein activity.

Some factors, linked to estrogen, and genetic (such as pregnancy and hormone therapy), phototoxic drugs, anticonvulsants, cosmetics, autoimmune thyroid diseases, and exposure to sunlight, among others, are involved in the pathogenesis of melasma, but the development of the condition cannot be traced to one of those factors alone.

Clinically, melasma presents three main patterns: centrofacial, malar, and mandibular. The centrofacial pattern is the most common; it affects the forehead, nose, upper lip, cheeks, and chin. Melasma is also classified according to its appearance under Wood’s light: epidermal, dermal, mixed, and undetermined. Epidermal lesions are accentuated when examined with the lamp, while an increase in dermal melanin becomes less evident under these conditions. This classification also has a prognostic impact: epidermal pigmentation is responsive to topical therapy and chemical peels, while dermal involvement complicates the treatment options. Finally, it is also important to classify melasma as transient or persistent: when the triggering stimulus is interrupted for one year and the melasma fades, it is classified as transient; otherwise it is considered persistent.

Given that it is a hard-to-treat disease that greatly influences the quality of life of affected individuals, a great number of studies have been conducted in an attempt to seek safe and effective therapies. The therapeutic approach includes constant photoprotection combined with topical compounds that inhibit tyrosinase, remove melanin and destroy melanin granules, thereby promoting a depigmenting action.

Hydroquinone is considered a first-line topical treatment; its whitening property was described in 1936 by Oettel. Its main function is to inhibit the production of tyrosinase, which prevents the conversion of DOPA into melanin; other actions, such as the degradation of melanosomes and the destruction of melanocytes, have also been proposed.

Due the great possibility of side effects, such as allergic contact or irritant dermatitis, post-inflammatory hyperpigmentation, permanent depigmentation, ochronosis, conjunctival melanosis, and nail depigmentation, alternatives such as azelieic, kojic acid, arbutin, licorice, emblica and belides extracts, niacinamide, mequinol, and Sepiwhite® chemical peels have proven to be safe and effective in whitening lesions and have few adverse effects. Studies have shown that combined therapies are preferred due to the synergism of substances and for reducing the side effects.

This study evaluated the efficacy and safety of a new kojic acid, arbutin, Sepiwhite® and Achromaxyl®-based cosmeceutical combination by comparing it to 2% and 4% hydroquinone in the treatment of facial melasma.

METHODS

This was a single-blind, comparative, monocentric study approved by the University’s Ethics in Human Research Committee and conducted according to the principles of the Declaration of Helsinki, the Research Good Clinical Practice, and the ANVISA’s (Brazilian national health surveillance agency) Resolution 196/96. This study included 120 female volunteers with epidermal or mixed facial melasma, with I to IV...
Fitzpatrick phototypes, aged 18-50.

To be included in the study, volunteers needed to be free of skin disease and habitual users of sunscreen (SPF ≥ 30). Exclusion criteria included the use of depigmenting products and/or having undergone cosmetic procedures in the 60 days prior to baseline.

After having read, understood, agreed, and signed a term of informed consent, study participants were divided into three groups: Group A (which used a thin layer of a kojic acid, arbutin, Sepiwhite® and Achromaxyl®- (Blancey®), Mantecorp Indústria Química e Farmacêutica Ltda., Rio de Janeiro/RJ, Brazil) based product twice a day, Group B (which used a thin layer of 2% hydroquinone (Clariderm®, Laboratórios Stiefel, Guarulhos/SP, Brazil) at night, and Group C (which used a thin layer of 4% hydroquinone (Solaquin®, Valeant Farmacêutica Ltda., São Paulo/SP, Brazil) at night. Daily photoprotection (Episol® Color SPF 30, Mantecorp Indústria Química e Farmacêutica Ltda., Rio de Janeiro/RJ, Brazil) was used in all groups. All groups used the products for 90 consecutive days.

The volunteers were clinically evaluated at baseline (D0) using Wood’s light. Their condition was quantitatively assessed using the Melasma Area Severity Index (MASI) and were photographed (Canon ™ Power Shot G10, Japan). The impact of melasma on patient quality of life was evaluated using the Melasma Quality of Life scale (MELASQoL). The volunteers were evaluated every 30 days (D30, D60, and D90).

The last visit (D90) included additional evaluations: effectiveness (rated as excellent, very good, good, fair, and none); tolerability (rated as excellent – absence of adverse events, good – adverse events were easily tolerated, average – tolerated adverse events did not lead to a discontinuation of use, bad – adverse events led to discontinuation of treatment); and overall performance of the treatment (assessed using the following metrics: zero = light melasma, 1 = almost clear with improvement of 90%, 2 = 75% improvement, 3 = 50% improvement, 4 = 25% improvement, 5 = no improvement, and 6 = worsening of the melasma).

RESULTS

The great majority of volunteers (102 – 85%) completed the study: Group A (34: five withdrew from the study for personal reasons and one was excluded due to diagnosis of depression without causal correlation to the use of the product), Group B (33: six withdrew for personal reasons and one presented acne, with a possible causal correlation to the use of the product), and Group C (35: two withdrew for personal reasons, one was excluded due to pronounced discomfort for one month, with a possible causal correlation to the use of the product, and one was excluded for having undergone a surgical procedure, without a causal correlation to the use of the product).

When asked about the possible etiology of their melasma, 58% of the volunteers thought pregnancy was the possible cause; 24% referred to hormonal contraceptives; 89% mentioned the sun; and 42% suspected genetic conditions. It is important to note that the volunteers could refer to more than one factor of correlation to melasma (Graph 1).

The MASI metric decreased throughout the study for all three groups, indicating statistically significant clinical improvement of melasma in all cases (comparing the MASI progression time point to time point for the three groups, the p-value was <0.001 for all comparisons): 1) Group A: initial average MASI was 12.73 (D0), rising to 10.89 (D30) and 9.65 (D60), with 8.63 (D90) at the end of the study. Group B had an initial average MASI of 14.04 (D0), falling to 11.90 (D30) and 10.00 (D60), with an average of 8.72 (D90) at the end of the study. Group C’s initial average was 11.73 (D0), falling to 10.01 (D30) and 8.29 (D60), with 7.04 (D90) at the end of the study (Table 1 and Graph 2).

When analyzing Group A versus Group B regarding the improvement in the MASI scale after 30 days, the p-value was 0.632, after 60 days the p-value was 0.642, and after 90 days p = 0.233. Likewise, when comparing Group A versus C, p = 0.620 was found after 30 days, p = 0.030 after 60 days, and p-value <0.001 after 90 days. The comparison between Groups B and C, in turn, yields a p-value = 0.931 after 30 days, p = 0.017 after 60 days, and p-value <0.001 after 90 days (Table 1).

The MELASQoL questionnaire attributes higher scores to greater degrees of personal dissatisfaction with melasma. In Group A, the average MELASQoL score on D0 was 45.62, falling to 28.14 on D90 (p-value <0.001). For Group B, it was 48.82 on D0, falling to 25.12 on D90 (p-value <0.001). Group C started at 48.49 on D0 and ended at 27.09 on D90 (p-value <0.001) (Table 2 and Figure 3). In the comparisons, Group A versus Group B yielded a p-value = 0.306; Group A versus C, p-value = 0.679; and Group B versus C, p-value = 0.507.

Regarding the item severity of use, in Group A, 67% of the volunteers presented no adverse events after 30 days of product use; 22% presented erythema, 6% desquamation, 3% tingling sensation, and 3% burning sensation. After 60 days of use 70% were asymptomatic, 16% presented erythema, and 14% scaling. After 90 days of use, 80% were asymptomatic, 14% presen-
Combined whiteners in melasma

Table 1: MASI scale descriptive statistics at baseline and after 30, 60, and 90 days of product use (Groups A, B, and C)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>MASI baseline</td>
<td>31,10</td>
<td>32,40</td>
<td>4% hydroquinone</td>
</tr>
<tr>
<td>MASI 30 days</td>
<td>27,50</td>
<td>27,50</td>
<td>2% hydroquinone</td>
</tr>
<tr>
<td>MASI 60 days</td>
<td>27,50</td>
<td>25,90</td>
<td></td>
</tr>
<tr>
<td>MASI 90 days</td>
<td>20,70</td>
<td>23,40</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12.73</td>
<td>10.89</td>
<td>10.65</td>
</tr>
<tr>
<td>Median</td>
<td>10.65</td>
<td>9.45</td>
<td>9.45</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>7.51</td>
<td>6.07</td>
<td>5.22</td>
</tr>
<tr>
<td>Minimum</td>
<td>3.30</td>
<td>2.40</td>
<td>5,13</td>
</tr>
<tr>
<td>Maximum</td>
<td>31.10</td>
<td>27.50</td>
<td>14.04</td>
</tr>
</tbody>
</table>

Graph 2: Progressive improvement in MASI in the three groups (p < 0.001)

DISCUSSION

Melasma is a melanodermia that primarily affects women and is very resistant to treatment, resulting in a considerable number of studies on its treatment. This dermatosis can negatively affect patients’ social and emotional balance, which can be evaluated with the MELASQOL questionnaire. It is worth noting that quality of life can improve with treatment. Among the therapeutic options are: topical depigmenters, lasers, intense pulsed light, chemical peels, and dermabrasion. Although hydroquinone is the most widely used depigmenting agent, it causes many adverse effects, which encourages the development of new products and new combinations of depigmenting agents to manage melasma.

The racial phenotypic differences related to cutaneous pigmentation are due to the melanocytes’ degree of activity, the quality of the melanosomes, the proportion and distribution of pheomelanin and eumelanin, and external factors such as UV radiation, which directly stimulate the production of melanin. The melanocytes’ activity can be influenced by the size of the melanosomes and the degree of enzymatic activity involved in the synthesis of melanin: fair skin melanosomes are smaller and found in clusters, and degrade in the middle malpighian layer, whereas in dark skin, they are larger and dispersed individually, and degrade slowly (thus allowing melanin granules to be found in the stratum corneum). Areas that are chronically exposed to the sun have a density of melanocytes up to twice that of non-photoexposed areas.

Tyrosine, an essential amino acid, is the starting element in the synthesis of melanin. In the presence of oxygen, tyrosinase oxidizes tyrosine and transforms it into DOPA, and then into dopaquinone. In the presence of cysteine (glutathione), the final product will be pheomelanin, an alkaline, yellowish pigment found in relatively high quantities in individuals with fair skin.

Conversely, in the absence of cysteine, dopaquinone is converted into dopachrome, which results in the formation of eumelanin, an alkaline, brownish pigment that is able to absorb and disperse UV radiation, thus reducing the harmful effects of the sun. Melanin has a high affinity with DNA; pheomelanin has the power to generate free radicals in response to sunlight, contributing to the toxic effect of solar radiation. This is why fair-skinned individuals have a higher risk of UV-induced epi-
dermal damage 7.

Hydroquinone is a phenolic derivative that competes with tyrosine as a substrate of tyrosinase, promoting damage to melanocytes and melanosomes 5. Prescribed concentrations range from 2–5%, and the efficacy and side effects are proportional to the strength 24. Side effects can be classified into acute (allergic contact or irritant dermatitis, post-inflammatory hyperpigmentation, and hypopigmentation) 15 or chronic (ochronosis, nail depigmentation, conjunctival melanosis, and corneal degeneration) 15. Phototype V and VI Individuals are more susceptible to such adverse events 21, 25. Since pregnancy is an important trigger for melasma, and hydroquinone cannot be used during pregnancy, natural treatment alternatives are very important 16, 21, 26.

In a 2009 study by Salem and colleagues, three groups of 15 patients with melasma, phototypes IV-V, were treated with 4% hydroquinone, 30% trichloroacetic acid peels, or frequency-doubled Q-switched Nd:YAG laser for six months. The hydroquinone treatment was the most effective (p <0.0001) 22. The frequency of adverse events with hydroquinone use 22 has given the pharmaco-cosmetic industry the incentive to search for alternative depigmenting substances with minimal side effects.

This study verified that the combination of kojic acid, arbutin, Sepiwhite® and Achromaxyl® is an excellent alternative in the management of melasma (Figures 1 and 2). From a clini-
cal point of view, a reduction in the severity of the lesions’ color intensity and size in the volunteers’ facial melasma could be observed through a 32% reduction in the MASI index (D90 versus D0). When comparing the combination of substances to 2% hydroquinone and 4% hydroquinone, a statistical difference was verified in Group C (4% hydroquinone, p-value <0.001), with no statistical difference in Group B (2% hydroquinone; p-value = 0.233). This finding positions the combination’s clinical potency between that of 2% hydroquinone and 4% hydroquinone.

The clinical benefits of the combination are linked to the ingredients’ action on diverse physiological stages of the melanin genesis. According to *in vitro* and *ex vivo* studies, the substances act to: inhibit the tyrosinase enzyme (by decreasing the biosynthesis of melanin and precursors), inhibit the gene expression and activity of endothelin-1 (by blocking the melanocytes’ dendriticity), reduce the gene expression and activity of PAR-2 (by impeding the transfer of melanin to the adjacent keratinocytes), and promote cell proliferation (by accelerating the elimination of melanin already deposited in the skin) 27.

Kojic acid [5-hydroxy-2-(hydroxymethyl)-4-pyrole] is an antimicrobial substance produced by bacteria and fungi (including some species of *Acetobacter*, *Aspergillus* and *Penicillium*) with chelating action over copper ions, resulting in the inactivation of tyrosinase and the inhibition of melanogenesis 28. Kojic acid has been successfully used as a depigmenter in the treatment of skin hyperchromias 1.

Alpha-arbutin is a depigmenting agent, known as 4-hydroxyphenyl-D-glucopyranoside 10 11. It is a hydroquinone glucoside, and thus affects melanogenesis 10 by inhibiting tyrosinase 11. The α-arbutin inhibits the tyrosinase more effectively than arbutin alone 12,23. The hydroquinone glucosides’ alpha-glycosidic linkages suggest an important role in the inhibition of tyrosinase 11. Its safety has been proven for cosmetic use 10. Polnikorn treated 35 cases of dermal or mixed melasma that had persisted for more than 6 months and were resistant to treatment with hydroquinone or Kligman’s formula; the study used applications of Q-switched Nd:YAG laser, at weekly intervals, and subsequently a 7% alpha-arbutin solution twice a day, combined with sunscreen. The results suggest that alpha-arbutin is an effective and safe alternative to hydroquinone in the management of melasma, and also minimizes hyperchromias resulting from light-based treatments and/or laser procedures to reduce the risk of melasma 23.

Sepiwhite® is a compound that contains phenylalanine (N-undecyl-10-enoyl-L-phenylalanine) 19, which is an amino acid that inhibits or activates Melanocyte-Stimulating Hormone (MSH) through its effects on the interaction of the
alpha-receptor's ligands \(^{15}\). Sepiwhite® acts as an antagonist of the MSH alpha-receptor, and has reduced the production of melanin in \(\textit{in vitro}\) tests \(^{19}\). A study carried out in 2009 by Bissett and colleagues noted that the use of the combination of 5% niacinamide and 1% N-undecylenoyl-phenylalanine was more effective than 5% niacinamide in isolation for reducing hyperpigmentation after 8 weeks of treatment, not only for melasma, but also for solar lentigines, ephelides, and lentigo senilis \(^{19}\). In a different randomized double-blind study (\(n = 30\): 28 women and 2 men, aged 47-75), 2% undecylenoyl-phenylalanine was effective and safe in the treatment of solar lentigines, presenting moderate improvement in 63.3% of cases and significant improvement in 36.6% \(^{29}\).

Achromaxyl® is an active principle composed of the \(\textit{Brassicaceae}\) family's fermented and hydrolyzed proteins. This substance reduces the amount of melanin by inhibiting tyrosinase activity, therefore causing the skin to whiten \(^{30}\). To satisfy increasingly demanding consumers, suppliers strive to develop products with more natural compounds – especially vegetable-based compounds that have been scientifically approved by clinical studies \(^{31}\).

Melasma negatively affects the quality of life of people with this condition, due to the personal dissatisfaction caused by its unattractive appearance and the effects on patients’ social lives \(^{7,32}\). MELASQoL captures this degree of dissatisfaction and measures the development of the condition during treatment \(^7\). The questionnaire assesses the conditions that are most affected by melasma, specifically social life, leisure, and emotional wellbeing \(^7\). The measurement evaluates items in ten domains: skin appearance, frustration, embarrassment, depression linked to the condition of the skin, the effects of the condition in relating to other people, the desire to be with other people, difficulty in demonstrating affection, not feeling attractive, feeling less important, and alterations in the patient’s sense of freedom \(^{32}\).

MELASQoL scores in this study demonstrated an improvement in quality of life for volunteers who used the combination of kojic acid, arbutin, Sepiwhite® and 40% Achromaxyl®; this improvement showed no statistical differences when compared to the two hydroquinone concentrations (Groups B and C). In the comparison between Group A and B, the p-value was 0.306, and between Group A and C, the p-value was 0.679. Therefore, the combination product is as effective as hydroquinone in improving the quality of life of patients with melasma. Studies of other cosmeceutical compounds – already analyzed by Costa and others – suggest that this category of products is of considerable importance not only in the clinical efficacy of melasma treatment \(^{18}\), but also in improving the quality of life \(^{30}\) of affected individuals. Such findings prove that these compounds are as effective as hydroquinone from both a clinical and quality of life perspective.
As discussed earlier, the use of hydroquinone presents a substantial risk of adverse events. In the present study, no definitive adverse events were observed in any of the groups except for the effects usually caused by depigmenting substances (pruritus, erythema, edema, burning sensation, tingling, and desquamation); erythema was the most prevalent. Those symptoms occurred in the beginning and decreased towards the end of the study; at D90, the absence of adverse events was statistically similar in all three groups (Group A versus B: p-value = 0.523; Group A versus C: p-value = 0.463, Group B versus C: p-value = 0.929).

**CONCLUSION**

Melasma causes an unattractive appearance and negative psychosocial effects. Although hydroquinone is the most widely used depigmenting agent, it has many undesirable effects. Therefore, several studies have evaluated the effectiveness of new depigmenting agents as alternatives to hydroquinone in the treatment of melasma. High levels of product safety, efficacy, and tolerability are of paramount importance for obtaining good results. In the present study, the use of a combination of kojic acid, arbutin, Sepiwhite® and Achromaxyl® was proven to be safe and effective in the treatment of melasma. Its clinical effectiveness was higher than that of 2% hydroquinone and lower than that of 4% hydroquinone, and it was as effective as hydroquinone in improving the quality of life of patients with melasma. Therefore, this product is an effective alternative for the treatment of melasma.