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# Evaluation of efficacy of cysteamine cream in the treatment of epidermal melasma: a randomized double blind placebo controlled study

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**Keywords:** Cysteamine hydrochloride, epidermal melasma, mexameter

**Running head:** Cysteamine cream for the treatment of melasma

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**Conflict of Interest:** Dr Behrooz Kasraee works at Scientis Pharma SA, but he did not have a role in data collection and analysis.

## Abstract

**Background:** Melasma is a difficult to treat hyperpigmentary disorder. While cysteamine is a known potent depigmenting agent, its efficacy in treating melasma has not been tested.

**Objective:** To study the efficacy of cysteamine cream in the treatment of patients with epidermal melasma.

**Methods:** In this double-blind randomized study, participating patients (n = 50) received either placebo (n = 25) or cysteamine cream (n = 25). Cysteamine cream or placebo were applied on the lesions once a day at bed time during four months. The efficacy of treatments was determined through Mexameter skin colorimetry, MASI score determination, Investigator's Global Assessment (IGA) and patient's questionnaires, all performed at the baseline, after two months and four months of treatment.

**Results:** Before commencing the treatments, mean difference between pigmented and normal skin (calculated by Mexameter®) was 75.2 ± 37 and 68.9 ± 31 in cysteamine and placebo groups respectively. After two and four months application of cysteamine and placebo cream, the mean differences were 39.7 ± 16.6 and 26.2 ± 16 in cysteamine group, and 63.8 ± 28.6 and 60.7 ± 27.3 in placebo group, respectively. Statistically significant differences were found between cysteamine and placebo group outcomes at both points ($p = 0.001$, $p = 0.0001$). At the end of the treatment, the MASI scores were significantly lower in the cysteamine group vs. placebo (7.2 ± 5.5 vs. 11.6 ± 7.9, $p = 0.02$). The Investigator Global Assessments

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and patients viewpoints indicated significant efficacy of cysteamine cream in contrast to placebo.

**Conclusion:** Cysteamine cream showed significant efficacy in the treatment of melasma.

**Key words:** epidermal melasma, cysteamine hydrochloride, mexameter, depigmenting agent, hydroquinone

## Introduction

Melasma is an acquired, chronic, recurrent, symmetrical hypermelanosis, which is characterized by brown patches of variable darkness on sun exposed areas of the body.[1, 2] Melasma is more common in women, accounting for 90% of all cases, and appears in all racial types, particularly those with skin type IV and V, residing in areas of high ultraviolet radiation.[1]

L-cysteamine is the product of L-cysteine metabolisation in human body.[3] This molecule acts as an intrinsic anti-oxidant and is known for its protective role against ionising radiation and as an anti-mutagenic agent.[3, 4]

Cysteamine hydrochloride (2-mercaptoethylamine hydrochloride) is also known as a potent depigmenting molecule for more than 4 decades. [4]

Cysteamine has been shown not to act through melanocytotoxicity, but via the inhibition of melanin synthesis to produce depigmentation.[5] The mechanism of inhibitory effect of cysteamine on melanin synthesis, however, is not yet throughly understood. Cysteamine is a thiol compound. Thiolic depigmenting agents are known to be inhibitors of tyrosinase and peroxidase, the two key enzymes involved in

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melanin biosynthesis.[6] Thiols are also known to scavenge DOPA quinone and remove it from the melanogenesis pathway.[7] Thiol molecules can act as chelators of Fe and Cu ions which are implicated in Fenton reactions involved in pigment synthesis.[8] Finally, cysteamine is shown to augment the intracellular levels of glutathione.[9] Higher levels of intracellular glutathione are associated with the shift of eumelanogenesis to pheomelanin synthesis; the latter being a much slower pathway than the former. This causes the melanogenesis process to proceed in a slower rate.[10]

In 1966 Chavin et al reported that the injection of cysteamine hydrochloride into the black goldfish skin causes skin depigmentation in this model.[4, 11] Later, Pathak et al reported the strong depigmenting effect of topical cysteamine hydrochloride in mammalian skin. Despite its strong depigmenting effect, cysteamine was never developed into a depigmenting product. The main reason was that cysteamine, as a thiol compound, has a very offensive odor which could not be covered by perfumes, prohibiting its use in topical preparations.[4]

A new technology has become recently available that permits to reduce the odor of cysteamine hydrochloride. This has permitted the use of cysteamine hydrochloride in topical depigmenting preparations. However, the depigmenting efficacy of the cysteamine cream which has become available as such has never been evaluated in a well-controlled study.

In the present study we evaluated the efficacy and safety of cysteamine cream for the treatment of epidermal melasma in a randomized, double blind, vehicle-controlled clinical trial.

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## Patients and Methods

### Study group

A randomized, double-blind, placebo-controlled parallel group study was conducted at two general hospitals (a university and a private hospital) and one research center. The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of Tehran University of Medical Sciences. The study protocol was registered in Iranian Registry of Clinical Trials (IRCT201212059800N2). Written informed consent was obtained from all patients prior to enrolment. The study started in April 2013 (first patient first visit) and ended in March 2014 (last patient last visit). Patients with facial melasma were recruited from the dermatology clinic of Imam Hospital (Tehran University of Medical sciences), the Skin and Stem cell Research Center as well as Bahman Hospital.

The treatment period was 4 months. Female and male patients aged 18-50 years with Fitzpatrick skin type III, IV and V were recruited to the study. The inclusion criteria were the diagnosis of epidermal melasma confirmed by Wood's lamp examination and minimum six months duration of the disease. None of the patients received any treatment for at least two months prior to the study.

The exclusion criteria were:

- Dermal and mixed type of melasma
- Receiving oral contraceptives at the time of the study or during the three months prior to it
- Topical or oral corticosteroid therapy
- Suffering from other pigmentation disorders

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- History of endocrine disorders
- Pregnancy and lactation

At the initial visit, patients' medical histories were taken, with particular focus on the time of onset of the melasma symptoms, history of pregnancy, contraceptive pill use, sun exposure, drug history, previous treatments for melasma, family history of melasma, and other influencing or exacerbating factors. The melasma severity was determined at the beginning of the study, and was categorized to four groups: (0) Minimal residual pigmentation; (1) Mild, slightly darker than the surrounding area; (2) Moderate, darker than the surrounding area; and (3) Severe, darker than the surrounding area. [1] This was only for determining the severity of melasma before treatment. The data were only used for descriptive analysis before treatment and not for comparison.

Patients were instructed to wash their face in the evening using the prescribed syndet bar and apply a thin layer of the cream (placebo or cysteamine) on their lesions 30 minutes later. It was recommended that they wash their face with tepid water 3 hours after the application of the cream. Neither the investigator nor the patients were aware of the identity of the treatments. All patients were provided with a broad-spectrum standard sunscreen with SPF 50+ and were instructed to apply it on their entire face and to repeat the application every 3 h during the day throughout the study period.

Cysteamine cream and the placebo (containing all ingredients except for cysteamine hydrochloride) were kindly provided by Scientis Pharma SA, Sharje SA, 23 Rue Joseph Girard, 1227 Geneva, Switzerland. They were sent to an independent investigator who did not have any role in the assessment of outcomes.

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Consecutive sampling method was used. The randomization sequences were generated by computer, blocked randomization was used by encoding tubes, 4 in each block. The sequences of the blocks were also generated by computer. In order to decrease the possibility of dislosure four labels for the creams were provided, two for placebo and two for drug.

Sampling and randomization were performed by an independent investigator. The investigators who assessed primary and secondary outcomes did not have a role in the randomization and allocation.

In order to assess the compliance of the patients, at each visit, the patients brought their tubes and the cream tubes were weighed at each follow-up appointment.

**Clinical assessment**

Clinical evaluations were performed by the same investigators at baseline, at month two and at the end of the study (month four). Standard facial images were obtained by skin surface analyzer (Visioface 1000D, CK electronic, GmbH, Germany) at baseline, two month's and 4 month's visits.

Evaluation of Melasma Area Severity Index (MASI) scores was performed at baseline, at month two, and at the end of the study. Briefly, according to the MASI score, the face is divided into four areas: forehead (F), right malar (RM), left malar (LM), and chin (C), which correspond to 30%, 30%, 30%, and 10% of the total face area, respectively. The melasma in each of these areas is graded on three variables. First, percentage of total area involved in each of these is given a numerical value: 0 (no involvement), 1 (0–9%), 2 (10–29%), 3 (30–49%), 4 (50–69%), 5 (70–89%), and 6 (90–100%). Second, darkness (D) is measured on a scale from 0 (absent) to 4 (maximum), and finally homogeneity is assessed on a scale from 0 (absent) to 4

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(maximum). The MASI score is then calculated according to Kimbrough-Green equation:[12]

$MASI = 0.3(D_F + H_F)A_F + 0.3(D_{RM} + H_{RM}) + 0.3(D_{LM} + H_{LM}) + 0.1(D_c + H_C)$ where D is darkness, H is homogeneity, A is area, F is forehead, RM is right malar, LM is left malar, C is chin, and the values 0.3, 0.3, 0.3, and 0.1 are respective percentages of the total facial area. Using this equation, the MASI scores can vary between 0 to 48. In this study, the mean of the MASI scores calculated by the same investigator was calculated for all patients.

Melanin content and erythema levels were calculated by Mexameter® (Mexameter® MX 18, MPA 9, Courage + Khazaka electronic, GmbH, Germany). Measurements were performed on the lesions and surrounding normal skin areas (at least four measurements were performed in each area and the mean of measurements were mentioned in analysis.

The differences in melanin values found in each lesion and the adjacent normal skin were calculated and the mean of the differences in each part of the face was used for the statistical analyses.

The investigator's global assessment (IGA) was performed according to Lee's scoring system [13]: (1) No effect (no visible changes of pigmentation), (2) Mild (decrease of visible pigmentation, but there is still some visible border), (3) Moderate (marked decrease of visible pigmentation, but there is still some visible border), and (4) Excellent (a complete loss of visible abnormal pigmentation).

The primary end-point in the trial registration document, was the investigators' assessment because the investigators did not trust on Mexameter® as a primary outcome. After starting the study the primary endpoint retrospectively has been changed, replacing the investigators' assessment with the Mexameter® evaluation.

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To determine the patients' viewpoints on the efficacy, patients were asked to select an item on the list that best described the effect of the cream on their melasma lesions (the list being the same as the investigator's assessment list).

The adverse effects, including erythema, dryness, itching, burning sensation, irritation and dyspigmentation (hypo or hyperpigmentation), were monitored during the study and were rated on a scale from 0 (none) to 3 (severe).

**Statistical analysis**

All analyses were performed using SPSS (statistical package for social sciences) software (version 16), Chicago, Illinois. Sample size was calculated using the expression below, whereby the one-sided type I error was set to $\alpha = 5\%$ and $\beta$ was equal to 0.2.

$$n = \frac{2(z_{1-\alpha} + z_{1-\beta})^2 p(1-p)}{(p_1 - p_2)^2}$$

The study was designed to detect a difference of 40% between an active treatment group (cysteamine) and the placebo group in the proportion of patients achieving a response according to Mexameter® data. Assuming proportions of 50% responders in the active treatment groups and 10% in the placebo group, a sample size of 18 patients per group was required to reach a power of 80%.

Assuming a dropout rate of < 10%, and accounting for an analysis following the intent-to-treat principle, 20 patients per group were required, however, we extended the sample size to 25 per group.

A p-value of 0.05 or less was considered to indicate statistical significance. For the comparison of MASI scores as well as Mexameter® measurements in each drug

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group, independent *t* test was used, as the data were found to be normally distributed. Normality was tested by Kolmogorov-Smirnov test and the grades in objective assessment were compared using Mann-Whitney test.

**Results**

After evaluating 70 patients for inclusion, 55 patients were recruited to the study, of which 53 completed the treatment until the follow up at four months (Figure 1). Two patients in the placebo group were decided not to continue their treatments without any specific reasons. These two patients were omitted from the study.

Baseline characteristics of the participants are summarized in Table 1.

In order to ensure the double-blind design, the data were encoded by an independent statistician.

The study population was randomly split into treatment and placebo groups, whereby 28 patients were treated with cysteamine cream and 25 were treated with the placebo. Three patients in cysteamine group were omitted from the study because of the adverse reactions and/or modification of the standard treatment in these patients. These three patients were followed up until the end of the study.

The age of the patients ranged from 23 to 50 years, with a mean of 39.4 ± 6. Moreover, 33 (62.3%) patients had Fitzpatrick skin type III and 20 patients (37.7%) had skin type IV. All the male subjects in this study had skin type IV.

The assessment based on the melasma severity scale revealed that 10 patients (20%) had mild melasma, 32 (64%) had moderate melasma, and 8 (16%) had severe melasma. The mean duration of the disease was 5.9 years (5.9 ± 4.4).

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Investigator's global assessment and patient's viewpoints on efficacy at the end of the study are mentioned in Table 2. Significant differences were found between placebo and cysteamine groups in both investigator's and patient's assessments (Mann-Whitney, 0.0001, 0.0001).

Before the treatments, the mean MASI scores in placebo and cysteamine group were 13 (13 ± 8) and 17.2 (17.2 ± 8.3), respectively, and the difference was not statistically significant (t test, CI 95%= -8.9 – 0.44).

The mean MASI scores reached 12.1 ± 8 after two months of treatment in the placebo group and 10 ± 6 in the cysteamine group. No significant difference was noted between the groups at this point (t test, CI 95%= -1.97– 6.1). At the end of the treatment period, the MASI score was 11.6 ± 7.9 in the placebo group and 7.2± 5.5 in the cysteamine group. The difference was statistically significant (t test, CI 95%= 0.5 – 8.3).

Using Mexameter®, the mean difference in melanin content of the lesions and the surrounding normal areas in the placebo and cysteamine groups were calculated, before treatment, and at month 2 and 4 (see Table 3). Statistically significant differences were found between the placebo and cysteamine groups at months 2 and 4.

The results of intention to treat analysis were the same as per protocol analysis.

Figure 2 shows a good clinical response in 3 melasma patients. According to the patients, they had already received several topical treatments (hydroquinone as well as hydroquinone free topical depigmenting creams) for their melasma without any improvements. Figure 3 also shows a good clinical response in a 43-year-old woman.

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Figure 4 shows the error bars of MASI scores and Mexameter® before, after two months, and after four months of topical treatment with cysteamine cream and placebo.

Table 4 shows the adverse events in placebo and cysteamine groups, indicating significant differences in erythema, dryness, itching, burning sensation and irritation reported in the two groups (Mann-Whitney, $p = 0.0001$, $p = 0.004$, $p = 0.001$, $p=0.001$, $p=0.002$).

Overall, 13 patients in the cysteamine group reported some degrees of adverse effects. The degree of adverse effects was rather mild in these patients, except for two of them who showed higher degrees of erythema and had to be treated with a topical corticosteroid for a few days. Once erythema and irritation were resolved, the treatment was re-initiated. Initially, the cream was applied for 10 to 15 minutes only, gradually increasing the contact time to the recommended 3 hours. With this method of gradually increasing the contact time, the cream was well tolerated and all the patients could resume the treatment until the end of the study (notably, these two patients were excluded from the study because of the modification of the standard treatment protocol). One of the cysteamine group patients who gave appropriate response to the treatment reported acne exacerbation after one month of therapy. Acne was controlled by the application of erythromycin 2% solution and the patient was excluded from the study. One female patient in the cysteamine group reported that the lesions had become darker after the first month of therapy. Mexameter® evaluations showed an overall increase in the melanin content of lesions as well as the surrounding normal skin (while the cream was only applied on the lesions and not onto the normal skin). The hyperpigmentation was resolved upon continuation of the treatment and a plausible effect was achieved at the end of the study.

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In the placebo group, only one patient complained from mild itching and burning sensation.

## Discussion

There are a wide range of depigmenting compounds used for the treatment of melasma.,[2, 14, 15] Some of these treatments include hydroquinone containing creams [16-18], azelaic acid preparations [19, 20], topical retinoids (e.g. retinoic acid)[12, 21], alpha-hydroxy acids [22, 23], kojic acid,[24, 25] and ascorbic acid,[26] responses to which are variable. Some treatments incorporate a combination approach such as triple-combination creams (hydroquinone, retinoic acid and a steroid).[27, 28] While some treatments may have significant side-effects, the chronic and relapsing nature of melasma is also an important issue, as any therapeutic results achieved might be difficult to maintain.[14]

In clinical practice, hydroquionone (HQ) containing products usually exhibit an acceptable efficacy, while most non-hydroquinone products show a rather weak anti-melasma effect, especially if used as monotherapy.

The depigmenting effect of HQ on human skin is most probably due to its melanocytotoxic potential. The cytotoxicity of HQ is not specific for melanocytes and other epidermal cells, such as keratinocytes, may also be affected. On the other hand, HQ is shown to induce mutations in *Salmonella* and in the Chinese hamster V79 ovarian cells. [29, 30]

The cytotoxic and mutagenic potentials of HQ have caused the drug regulatory authorities of several countries to ban the use of this compound as a depigmenting agent. Following the ban of HQ, the lack of a safe and effective depigmenting

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compound has caused many investigators to search for a replacement for hydroquinone.

Cysteamine (β-mercaptoethylamine hydrochloride; Sigma-Aldrich Co. LLC) is a non-melanocytotoxic[31] and non-mutagenic molecule that has shown a strong depigmenting action in different animal models[11]. Frenk et al used topical cysteamine on black guinea pig skin in comparison with 4% hydroquinone and showed that cysteamine is more potent than hydroquinone in producing skin depigmentation. [32]

Qui L, et al showed that cysteamine (and its oxidized monomer cystamine) regulate melanization in viable melanocytes, confirming melanogenesis inhibition, and not melanocytotoxicity, as the mechanism of depigmenting action of cysteamine.[5]

Cysteamine is known as an anti-carcinogenic compound.[31] It is shown to act as an anti-mutagenic molecule by Hoffmann et al.[33]

Despite the high safety and efficacy profiles of cysteamine, this molecule was not managed to be developed to a topical depigmenting product for human use, due to its very offensive odor especially in cream conditions. In the present study, the effectiveness of cysteamine cream in the treatment of melasma has been assessed. Despite the high clinical efficacy of cysteamine in our study, the MASI scores as well as the Mexameter® results did not indicate a 100% disappearance of melasma lesions. It should be noted, however, that many of our patients had recalcitrant melasma and were mostly “unresponsive” to other forms of treatments including hydroquinone.

Overall, the adverse effects in cysteamine group were more pronounced when compared to the placebo group. However, most of the adverse effects were transient and were significantly reduced after about one week of the initiation of treatment and

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gradually disappeared. This outcome indicates that cysteamine is generally well tolerated. Some of our patients complained that the cream had a residual sulphur-like odor. This, however, was considered as tolerable by these patients. It should be noted that the sulphur smell reported by some patients might have interfered with the blindness of the investigators and this was a limitation factor in our study. Only a few patients reported this odor.

The other limitations of the present study are the lack of histological assessments as well as quality of life evaluations in our patients. The systemic absorption of cysteamine was not assessed in this study.

In conclusion, the treatment with cysteamine cream met the primary outcome of decreasing melanin content of the lesions after two and four months of application, demonstrating its efficacy in epidermal melasma. Literature review indicates that the present study might be the first controlled clinical trial that examined the efficacy of cysteamine cream in the treatment of a hyperpigmentary disorder in human.

Hopefully, clinical trials would be conducted in near future in order to determine the depigmenting efficacy of cysteamine in comparison with other known skin depigmenting compounds in human.

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## Figure legends

Figure 1:

Flow diagram according to CONSORT guidelines showing the flow of participants in the trial, including dropouts.

Figure 2:

A 37 years old woman with 5 years melasma duration, A, before treatment, B, after 4 months treatment by topical cysteamine cream; a 37 years old woman with 5 years duration of melasma, C, before treatment, D, after 4 months application of

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cysteamine cream; a 36 years old woman with3 years duration of melasma, E, before treatment and F after 4 months treatment by topical cysteamine.

Figure 3:

A 43 years old woman with 10 years melasma, A, C, before treatment, B, D, after 4 months treatment by cysteamine cream.

Figure 4:

Error bar of MASI scores before (A), after 2 months therapy (B), and after 4 months treatment (C); Mexameter differences before (D), after 2 months (E), and after 4 months treatment (F).

Table 1- Baseline patient characteristics

| **Variable** | **Cysteamine (n=28)** | **Placebo( n=25)** |
|---|---|---|
| **Age (years), mean ± SD** | 39.9 ± 6.6 | 39.7 ± 5.6 |
| **Sex, n (%)** | | |
| **Male** | 4 (14.3) | 3 (12) |
| **Female** | 24 (85.7) | 22 (88) |
| **Skin Type** | | |
| **III** | 19 (67.9) | 14 (56) |
| **IV** | 9 (32.1) | 11 (44) |
| **Duration of melasma (years), mean ± SD** | 5.9 ± 3.4 | 6.2 ± 5.3 |

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| | | |
|---|---|---|
| **MSS** | | |
| **Minimal residual pigmentation** | 0 | 0 |
| **Mild** | 4 (14.3) | 6 (24) |
| **Moderate** | 18 (64.3) | 17 (68) |
| **Severe** | 6 (21.4) | 2 (8) |
| **MASI Scores** | | |
| **(mean ± SD)** | 17.2 ± 8.1 | 13 ± 8.1 |
| **Mexameter findings** | | |
| **(mean ± SD)*** | 81.5 ± 41.8 | 68.9 ± 31 |

MSS, Melasma Severity Score, MASI, Melasma Area Severity Index

* mean: mean difference between lesion and surrounding normal skin

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Table 2- Hypopigmentation grading in placebo and cysteamine groups assessed by investigators

| | Investigator's global assessment | | | | Patient's viewpoint on efficacy | | | |
|---|---|---|---|---|---|---|---|---|
| **Hypopigmentation Grading** | **Placebo** | | **Cysteamine*** | | **Placebo** | | **Cysteamine*** | |
| | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| **1** | 12 | 48 | 0 | 0 | 7 | 28 | 2 | 8 |
| **2** | 12 | 48 | 6 | 15.8 | 17 | 68 | 7 | 28 |
| **3** | 1 | 4 | 19 | 84.2 | 1 | 4 | 15 | 60 |
| **4** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| **Total** | 25 | 100 | 25 | 100 | 25 | 100 | 25 | 100 |

and by the patients.

(1) no effect (no visible changes of pigmentation); (2) mild (decrease of visible pigmentation, but there is still some visible border); (3) moderate (marked decrease of visible pigmentation, but there is still some visible border); and (4) excellent (a complete loss of visible abnormal pigmentation). *A significant difference was found between placebo and cysteamine groups by investigator's global assessment and also by patient's viewpoint on efficacy (Mann Whitney, P=0.0001, P=0.0001)

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Table 3- Mean differences of pigment content of the lesions and normal skin using Mexameter before, after 2 months and at the end of the study at month 4.

| | | Mexameter | | |
|---|---|---|---|---|
| | **N** | **Before Treatment** | **After 2 Months** | **After 4 Months** |
| **Placebo** | 25 | 68.9 ± 31 | 63.8 ± 28.6 | 60.7 ± 27.3 |
| **Cysteamine** | 25 | 75.2 ± 37 | 39.7 ± 16.6 | 26.2 ± 16 |
| **P value** | | 0.51 | 0.001 | 0.0001 |
| **CI 95%** | | -25.8 – 13.01 | 10.8 – 37.4 | 21.8 – 47.2 |
| | | | | |
| **Placebo** | 25 | 68.9 ± 31 | 63.8 ± 28.6 | 60.7 ± 27.3 |
| **Cysteamine** | 28 | 81.5 ± 41.9 | 43.6 ± 20.2 | 26.7 ± 16.3 |
| **P value** | | 0.22 | 0.004 | 0.0001 |
| **CI 95%** | | -33.1 – 7.9 | 6.6 – 33.7 | 21.7 – 46.2 |

The upper part shows the placebo and cysteamine group when the 3 patients with adverse events (who modified their treatment) were omitted and the lower part shows the results when they were included in the analysis.

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Table 4- Adverse events reported in the placebo and cysteamine groups.

| | | | Placebo | | | Cysteamine | |
|---|---|---|---|---|---|---|---|
| | **Grade** | | **N** | **%** | | **N** | **%** |
| **Erythema** | **0** | | 25 | 100 | | 15 | 53.6 |
| | **1** | | 0 | 0 | | 5 | 17.9 |
| | **2** | | 0 | 0 | | 3 | 10.7 |
| | **3** | | 0 | 0 | | 5 | 17.9 |
| **Dryness** | **0** | | 25 | 100 | | 20 | 71.4 |
| | **1** | | 0 | 0 | | 5 | 17.9 |
| | **2** | | 0 | 0 | | 3 | 10.7 |
| | **3** | | 0 | 0 | | 0 | |
| **Itching** | **0** | | 24 | 96 | | 16 | 57.1 |
| | **1** | | 1 | 4 | | 6 | 21.4 |
| | **2** | | 0 | 0 | | 6 | 21.4 |
| | **3** | | 0 | 0 | | 0 | 0 |
| **Burning** | **0** | | 24 | 96 | | 16 | 57.1 |
| | **1** | | 1 | 4 | | 4 | 14.3 |
| | **2** | | 0 | 0 | | 8 | 28.6 |
| | **3** | | 0 | 0 | | 0 | 0 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Irritation** | **0** | | 25 | 100 | | 19 | 76.9 |
| | **1** | | 0 | 0 | | 1 | 3.6 |
| | **2** | | 0 | 0 | | 6 | 21.4 |
| | **3** | | 0 | 0 | | 2 | 7.1 |
| | | | | | | | |
| **Hyperpigmentation** | **0** | | 25 | 100 | | 27 | 96.4 |
| | **1** | | 0 | 0 | | 1 | 3.6 |
| | **2** | | 0 | 0 | | 0 | 0 |
| | **3** | | 0 | 0 | | 0 | 0 |

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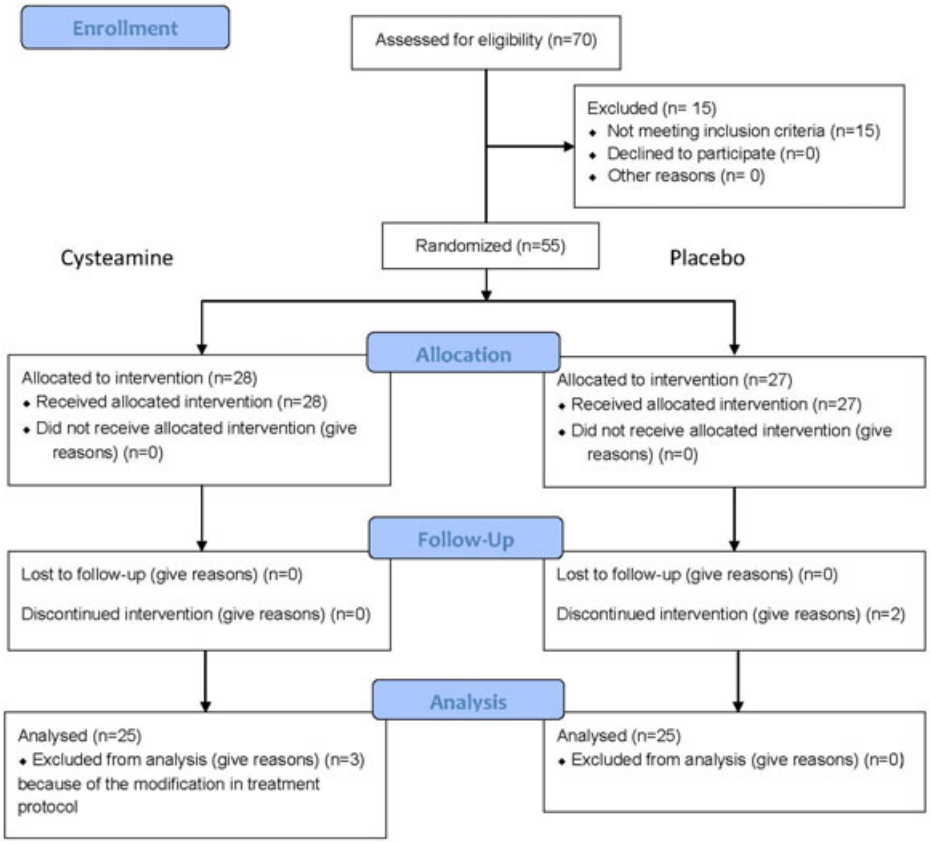


Fig 1- Flow diagram according to CONSORT guidelines showing the flow of participants in the trial, including dropouts.

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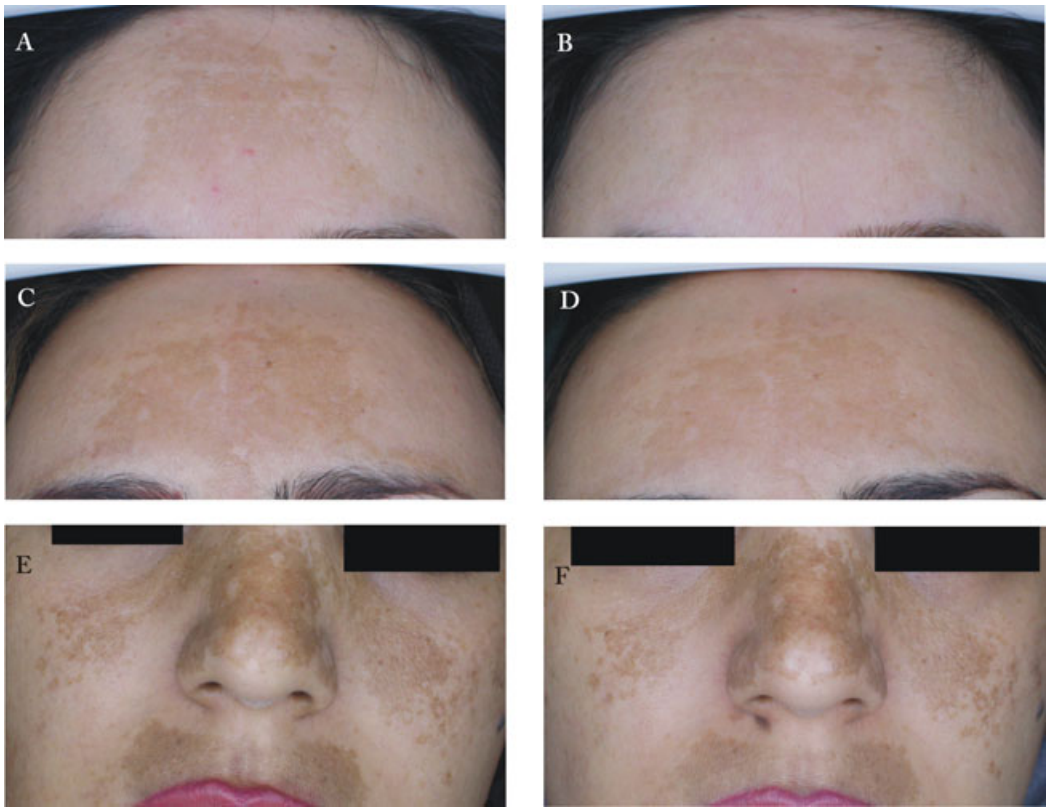

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