Efficacy of Topical Silymarin in Melasma Treatment During Pregnancy and Breastfeeding

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EFFICACY OF TOPICAL SILYMARIN IN MELASMA TREATMENT DURING PREGNANCY AND BREASTFEEDING

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

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ABSTRACT

Melasma is a common hyperpigmentation disorder that negatively impacts quality of life. Melasma predominatey affects women of reproductive age and is commonly acquired during pregnancy. Current treatment options focus on prevention with limited therapies available once melasma has developed for patients who are pregnant or breastfeeding. One potential therapy that has shown promise for the treatment of melasma is silymarin, a derivative of milk thistle. However, the effect of silymarin on melasma in the pregnant and breastfeeding population has never been investigated. This double-blind randomized placebo-controlled trial will investigate whether topical silymarin can improve the severity of melasma in pregnant and breastfeeding women. Outcomes will be measured using the Melasma Area Severity Index to assess changes from baseline. The results of this study will help inform providers about the use of silymarin for melasma management during pregnancy and may provide an additional therapy option.
CHAPTER I: INTRODUCTION

1.1 Background

Melasma is a commonly acquired hyperpigmentation disorder of the skin characterized by symmetric brown to gray macules and patches that primarily occur on sun-exposed areas of the face\(^1\). These patches can affect the forehead, cheeks, chin, and are classified as either having a centrofacial, malar, or mandibular pattern, respectively\(^1,2\). Of these patterns, centrofacial is the most common, comprising 50-80% of cases\(^3\). The hyperpigmented patches are a result of excess melanin deposition by hyperactive melanocytes. Thus, this disorder is also classified histologically by the depth of melanin deposition into the epidermis, the dermis, or a mixture of both layers of the skin\(^4\).

Patients most at risk for developing melasma are women of reproductive age, with the average age of onset between 20-30 years old\(^5\). Although its pathogenesis is not fully understood, key factors that affect melasma’s development include family history, skin type, sun exposure, and hormonal influences\(^3\). In several studies, individuals with intermediate skin types, or Fitzpatrick skin types III-IV, accounted for more than 70% of patients with melasma\(^6-8\). These epidemiological studies also showed melasma developed during pregnancy for more than 23% of cases, suggesting a hormonal trigger for melasma\(^9,10\).

Melasma is often referred to as “the mask of pregnancy” because the prevalence is between 10% to 50% amongst pregnant women\(^11-13\). Pregnancy is also often one of the most-cited triggering factors by patients\(^5\). Higher levels of estrogen, progesterone, and melanocortin found particularly during the third trimester of pregnancy are thought to contribute to melasma’s development\(^5\). These elevated hormones stimulate
melanogenesis, leading to the development and deposition of pigment in the skin. Pregnancy-induced melasma is also associated with more facial area involvement than melasma developed outside of pregnancy. Although melasma can be triggered by various factors, it is estimated that over 5 million people in the United States are living with these hyperpigmented facial patches and its known detrimental effects on quality of life\(^\text{14}\).

Like other skin diseases such as acne or psoriasis, melasma bestows a psychological burden on those affected\(^\text{15}\). These highly visible hyperpigmented areas on the face often result in depression, embarrassment, and frustration in patients with melasma\(^\text{16}\). Patients reported a negative influence on social relationships and work productivity with feelings of low self-esteem, shame, and dissatisfaction. Studies show decreased quality of life in melasma patients compared to patients with other pigmentation disorders, such as vitiligo\(^\text{17}\). Furthermore, studies examining the relationship between severity of melasma and effect on quality of life have shown no association, suggesting that even a small amount of melasma may place significant emotional distress on a patient\(^\text{18}\). Therefore, melasma cannot be dismissed as a benign cosmetic condition. It is crucial to treat melasma as it develops to prevent its negative consequences on quality of life.

Treatment for melasma depends on severity of disease, with therapies targeting different pathogenic factors such as abnormal pigmentation and photodamage. Photoprotection, including sunscreen and sun avoidance, is essential in melasma management. If used correctly, sunscreen alone has been shown to prevent melasma development, reduce melasma severity, and improve quality of life\(^\text{19,20}\). Thus, the use of broad-spectrum sunscreen is fundamental to any melasma treatment regimen. In addition
to sunscreen, topical lightening agents are considered first-line therapy. Topical hydroquinone is the treatment of choice for mild, moderate, and severe melasma. Hydroquinone significantly improves melasma by inhibiting tyrosinase, a key enzyme used in melanin production\textsuperscript{21}. When hydroquinone is combined with tretinoin and dexamethasone, the resulting triple combination cream is first-line treatment for moderate to severe disease due to its success in improving or clearing melasma in 60-80\% of patients\textsuperscript{1}. Second-line therapies for melasma include chemical peels and oral tranexamic acid. Chemical peels use a variety of acids, most commonly glycolic acid, to increase skin turnover. However, pigmentation improvements from chemical peels are only temporary since these acids do not affect melanogenesis. Similarly, although tranexamic acid is effective in treating melasma, relapses occur once oral therapy is discontinued.

Treatment for melasma remains a challenge due to its chronic and relapsing nature, prompting the search for alternative therapies. Newer therapies for melasma treatment include non-hydroquinone topical agents such as azelaic acid, kojic acid, niacinamide, and silymarin\textsuperscript{22}. These agents are as effective as hydroquinone at reducing melasma severity and are considered first-line alternatives in patients who cannot tolerate hydroquinone use. However, many of these non-hydroquinone options still may cause side effects. Azelaic acid can cause burning, stinging, and pruritus while kojic acid is reported to cause contact dermatitis and mutagenicity\textsuperscript{22}. On the other hand, the non-hydroquinone agents with no reported adverse effects, such as silymarin, have limited studies available and have not been tested in certain populations. Therefore, when choosing a treatment option, various factors must be considered including effectiveness, safety, tolerability, and individual patient characteristics such as pregnancy. In pregnant
women, overall therapy options are limited despite greater risk for melasma development. Many treatments for melasma do not consider use during pregnancy with virtually no clinical trials targeting this population. This demonstrates a gap in the literature and a need for additional studies.

1.2 Statement of the Problem

Melasma most commonly affects women of reproductive ages. Despite its known detrimental effects on quality of life, limited options are available for melasma management during pregnancy and breastfeeding. The current standard of care during this period focuses on prevention using sunscreen and sun-avoidance. However, once melasma has developed, few treatment options are available that do not carry a financial burden or risks to the pregnancy itself. Hydroquinone, the first-line agent for melasma treatment, is a category C for pregnancy and results in about 35% secretion in breast milk. Even second-line therapies such as tranexamic acid may carry more risks during pregnancy. Tranexamic acid is labeled for use as an antifibrinolytic agent. It works by preventing the breakdown of blood clots to reduce the risk of bleeding. Therefore, its use during pregnancy, a hypercoagulable state, may increase the risk for adverse events such as thrombosis or embolism. As for laser modalities and chemical peels, these often bestow a financial burden. Thus, pregnant patients may defer these options to prepare for the expense of a newborn. As for the novel non-hydroquinone agents, many have never been studied for use during pregnancy with little to no data available on their safety profile in human subjects. One of these agents is silymarin, a pregnancy category B medication. Despite promising results in reducing melasma
severity and little to no side effects with use, studies investigating silymarin’s effectiveness have focused solely on non-pregnant individuals. Because pregnancy is known to trigger melasma, finding viable treatment options for use during pregnancy and breastfeeding to combat the psychosocial issues associated with melasma is important.

1.3 Goals and Objectives

The primary goal of this study is to investigate the efficacy of topical silymarin cream for melasma treatment in pregnant and breastfeeding women. The proposed study will use a randomized placebo-controlled trial to establish silymarin’s role in melasma treatment within the pregnant population. Efficacy will be determined by the mean reduction in melasma severity from baseline using the Melasma Area and Severity Index (MASI) validated by Pandya et al\textsuperscript{25}. Secondary outcomes of this study will evaluate changes to participants’ quality of life before and after treatment.

1.4 Hypothesis

In pregnant and breastfeeding women with melasma, daily treatment with 1.4% topical silymarin will show a statistically significant mean difference in Melasma Area Severity Index (MASI) score from baseline to follow-up at 45 and 90 days compared to placebo.

1.5 Definitions

- Postpartum: Beginning after delivery of infant and ending six to eight weeks after when the effects of pregnancy on many body systems have returned to the pre-pregnancy state.
• *Breastfeeding:* The action of feeding an infant with milk from the breast. For our study, when referring to breastfeeding women, we only include women in the postpartum period (see definition above).
1.7 References


CHAPTER II: REVIEW OF THE LITERATURE

2.1 Introduction

An extensive literature search of multiple databases, including PubMed, Cochrane, and Ovid Medline, was conducted between July 2021 and May 2022. Articles were found using various combinations of the following keywords: melasma, chloasma, melanosis, melanin, pregnancy, pregnancies, breastfeeding, lactation, silymarin, milk thistle, hyperpigmentation, treatment, safety, efficacy, sunscreen, MASI, quality of life, MELASQOL. Additional articles were taken from the references of these studies if relevant to our proposed study. All articles were filtered for English language then reviewed for significance. Studies exclusively looking at melasma in men were excluded.

2.2 Review of Relevant Studies

2.2.1 Melasma and Pregnancy

Multiple studies have demonstrated the prevalence of melasma during pregnancy. In a cross-sectional study of 905 pregnant women, 54% of them reported developing melasma while pregnant. This prevalence was similar to other reported findings of melasma in 46% to 70% of pregnant women. However, some studies have reported differences depending on geographic location. A study of southern Brazilian women found melasma in 11% of the pregnant population while a study of 400 pregnant women in Iran found melasma in 16%. Despite the differences in prevalence among different ethnic groups, these numbers still suggest that pregnancy is a trigger for the development of melasma.
The pathogenesis for pregnancy-induced melasma is unknown. One triggering factor thought to play a role in inducing hyperpigmentation changes is the increase in circulating hormone levels found during this period. The placental hormones and lipids along with ovarian hormones may upregulate melanin synthesis. The increased levels of progesterone and estrogen found during the end of the first trimester and throughout the second trimester synergistically stimulate melanogenesis. Estrogen stimulates the production of enzymes including tyrosinase, the enzyme involved in the first step of melanin synthesis. Progesterone’s role in melanogenesis is less clear, but it may be involved in stimulating epidermal melanocytes. When examining biopsies of melasma lesions compared to healthy skin biopsies, immunohistochemical stains revealed increased progesterone receptors in the epidermal layer of the skin and increased estrogen receptor expression in the dermis. These findings suggest a role for both estrogen and progesterone in the development of melasma.

2.2.2 Treatment for Melasma During Pregnancy

Current treatment for melasma during pregnancy focuses primarily on preventive measures such as sunscreen use and sun avoidance. UV light exacerbates hyperpigmentation conditions like melasma, so photoprotection with sunscreen use cannot be overlooked. Multiple studies have shown the importance of using sunscreen to maximize outcomes of patients with melasma. One study examining the effects of sunscreen use alone on melasma and quality of life improvement showed sunscreen significantly improved both MASI and MELASQOL scores after 12 weeks of proper application. In a study looking at its use in pregnancy, Lakhdar et al evaluated the role
of SPF 50+ sunscreen on preventing and treating melasma in 200 pregnant women. It found that proper application of sunscreen every 2 hours helped prevent melasma development or worsening in 180 pregnant women out of 185 that completed the study. The study also recorded pigmentation improvement in a few participants with pre-existing melasma. Although limited by lack of a control group, the results of this study help demonstrate the benefit of sunscreen in melasma prevention and its fundamental role in the treatment of melasma.

Apart from sunscreen, other agents are often deferred as many treatments for melasma have not been assessed for safety during pregnancy and breastfeeding. Our literature search yielded only one clinical study investigating a non-sunscreen treatment agent for use during pregnancy. This double-blind randomized clinical trial evaluated the efficacy of topical aloe vera in improving melasma in a sample of 180 pregnant women. Gharfarzadeh and Eatemadi found a significant difference in mean MASI score in the intervention group compared to controls. A major limitation of this study is that they did not control for sunscreen use in participants. As mentioned previously, sunscreen is a fundamental part of any melasma treatment regimen as it can prevent hyperpigmentation worsening. Without controlling for sunscreen use, it is unclear whether the control group’s non-improvement in melasma is due to the lack of intervention, the lack of sunscreen, or both. Despite this limitation, this study has many strengths including a large sample size, good randomization, and its novelty in using a pregnant population.
2.2.3 Mechanism of Action of Silymarin

Silymarin is derived from the seeds of the milk thistle plant, *Silybum marianum*. Silybin, the active component of silymarin, has been used for its antioxidant and anti-inflammatory properties for centuries\(^1\). One of its first and widely studied uses is as a hepatoprotective agent for liver diseases such as cirrhosis and hepatitis. However, silymarin use has expanded beyond hepatoprotection to include utilization in protection of the skin, heart, and nervous system\(^1\). To understand the versatility of silymarin for skin protection, it is important to understand its effects at a cellular level.

In dermatology and cosmetics, silymarin is used for its antioxidant effects and its ability to protect from damaging ultraviolet (UV) light\(^1\). Normally, UV light causes the formation of reactive oxygen species (ROS) which can modify proteins and activate enzymes within the skin. These activated enzymes break down cellular components including collagen and elastin, leading to decreased skin hydration and wrinkle formation. However, antioxidants such as silymarin act as free radical scavengers to neutralize ROS and prevent premature aging. In addition to neutralizing free radicals, silymarin directly protects the skin by absorbing UV rays and prevents the breakdown of collagen by inhibiting the enzyme collagenase\(^1,16\).

Along with its ability to reduce damage done by solar radiation, silymarin can alter pigmentation production within the skin by indirectly inhibiting melanogenesis\(^1\). Choo et al. found silymarin reduces the expression of tyrosinase, a key enzyme in melanin synthesis\(^1\). Furthermore, this inhibitory effect was found to be dose-dependent without associated cytotoxicity at higher concentrations of silymarin. These findings suggest a role for silymarin as a skin-lightening agent.
These studies examining the properties of silymarin suggest a promising role in the treatment of melasma. The photoprotective properties of silymarin may prevent worsening of melasma by UV radiation while the depigmenting properties may target the hyperpigmentation patches characteristic of melasma.

2.2.4 Silymarin Effect on Melasma

Our literature search yielded a total of 3 studies assessing the effect silymarin had on melasma, demonstrating the need for further research on this topic. The first study performed enrolled 96 patients randomized into three groups: silymarin 7mg/ml, silymarin 14mg/ml, and placebo. At the end of the four-week study, the size of the melasma lesion was significantly reduced in both silymarin groups but not the placebo group. Additionally, the group using the higher strength 14 mg/ml silymarin showed faster response to treatment than the 7 mg/ml group, suggesting silymarin improves melasma in a dose-dependent manner. A strength of this study was that it was the first randomized clinical trial to evaluate silymarin for use in melasma treatment. Other strengths included its double-blind design with use of a placebo to blind participants to the intervention. A limitation of this study was the duration of treatment was only one month with no follow-up performed.

A comparative study by Nofal et al examined the efficacy and safety of topical silymarin when compared to hydroquinone for melasma treatment. It was found that MASI score significantly decreased in all treatment groups with no differences in patient satisfaction between groups. However, hydroquinone had significantly more reported adverse effects including erythema, burning, and scaling while silymarin had none. It was
concluded that silymarin may provide a safer and as effective alternative to hydroquinone. Strengths of this study included its novelty in comparing silymarin to current standard of care and its use of follow up to evaluate melasma recurrence after treatment ended. Limitations included a small sample size of 42 female patients who were unable to be blinded to treatment groups due to differences in applications of silymarin and hydroquinone.

The most recent study was a randomized clinical trial done in 2021 to compare topical silymarin to low fluence laser therapy for the treatment of melasma. Melasma improved significantly in both groups before and after treatment with no difference between groups in the percentage of change. The only difference occurred with reported side effects. Almost all patients in the laser group recorded edema and erythema after treatment while one patient in the silymarin group reported worsening of melasma. However, this worsening was found to be due to lack of sunscreen adherence per protocol.

Although limited studies are available on the use of topical silymarin for melasma treatment, the above studies showcase the promising results of silymarin. Not only is it effective at reducing melasma lesions, but it may provide a safer alternative to conventional treatments such as hydroquinone and laser modalities. However, the lack of additional studies and the limited group sizes in the current studies indicate a need for further investigation.
2.3 Review of Studies Analyzing Possible Confounders

Confounding variables threaten the validity of studies. Like previous studies investigating treatments for melasma, there are several confounders that need to be addressed in our study such as sun exposure, thyroid disorders, and family history.

Sun exposure is known to trigger melasma development and worsen existing hyperpigmentation\(^{20,21}\). Studies have addressed the level of sun exposure in their subjects in various ways. Most studies simply recorded participants’ estimations of average daily sun exposure at baseline or documented whether a participant’s occupation or hobbies led to extended time outdoors\(^{11,19,22}\). Another study had participants self-report frequency of sun exposure throughout the duration of the study\(^{12}\). Regardless of the method used to record sun exposure, almost all studies required subjects to use sunscreen and limit time in the sun if possible.

Other potential confounders are comorbid conditions such as thyroid disorders. A study by Cakmak et al. found thyroid-stimulating hormone (TSH) levels to be significantly higher in patients with melasma when compared to matched controls\(^{23}\). In addition to TSH, differences in other thyroid related components such as anti-TPO and antithyroglobulin antibody were found between melasma patients and controls\(^{24}\). Like many of the reviewed studies, our proposed study will address this confounder by excluding any participant with known thyroid dysfunction.

Another variable to consider is genetics including family history and Fitzpatrick skin type. A positive family history of melasma can vary from 18% to almost 50% of patients\(^{25,26}\). Epidemiological studies have also found that individuals with Fitzpatrick skin types III or IV made up more than 70% of patients with melasma\(^{27-29}\). These studies
suggest individuals with darker skin types and a positive family history are more likely to develop and have melasma than those with lighter Fitzpatrick skin types. Our study will survey participants at baseline on these confounders and attempt to control for these differences through randomization of our sample.

Lastly, as discussed earlier in this chapter, hormones can affect the progression of melasma. Other studies have addressed this confounder by simply excluding any pregnant or nursing individuals\textsuperscript{18,19,22}. However, because our study is exploring treatment effects specifically in a pregnant and breastfeeding population, taking a similar approach as other studies is impossible. We aim to address potential hormonal effects of pregnancy in our sample size calculation (see Chapter 2.4.6). Besides pregnancy, another source of hormonal influence is oral contraceptive pills (OCPs). In epidemiological surveys, contraceptive use is often one of the commonly cited causes of melasma development following sun exposure and pregnancy. In a survey of 148 women who used OCPs, 25\% of them reported melasma onset after its use\textsuperscript{21}. Our study will limit this confounder by excluding postpartum women taking OCPs.

2.4 Review of Relevant Methodology

2.4.1 Study Design

The proposed study will be a two-arm, double-blinded, placebo-controlled, randomized clinical trial evaluating the efficacy of topical 1.4\% silymarin for melasma treatment in pregnant and breastfeeding women. The empirical studies presented above used randomized clinical trials to test silymarin’s efficacy; however, only one utilized a double-blind design. The studies done by Nofal et al and Ibrahim et al were not able to
blind their participants to the treatment received as their non-silymarin comparison had a different method of administration\textsuperscript{19,22}. This makes it apparent to the participants which group they have been randomized to, introducing a source of bias into these study designs. Our study will address this issue using a placebo with the same instruction methods for application as the intervention arm. Another critique of these prior studies has been their small sample size. The number of participants per treatment group in these studies ranged from 14 to 32, demonstrating the need for studies with larger sample sizes.

### 2.4.2 Study Population and Selection Criteria

The study population from which subjects will be recruited consists of adults with melasma who are pregnant or breastfeeding. The subjects will be recruited from outpatient dermatology practices and obstetric centers within the Yale New Haven Health system. By recruiting from obstetric centers where women are receiving prenatal and postpartum care, we aim to increase the generalizability of our study by including participants who would not otherwise present for dermatologic care.

Our study will only include women who are in their second trimester or later of pregnancy or who are no more than 6 weeks postpartum and breastfeeding. The choice to include only women in their second trimester of pregnancy or later is based on the study done by Ghafarzadeh and Etemadi, which is one of the only clinical trials to evaluate a topical agent for treatment of melasma during pregnancy\textsuperscript{13}. Additionally, the placental and ovarian hormones that are believed to stimulate pregnancy-induced melasma become elevated in the second half of pregnancy\textsuperscript{6,8}. As for including breastfeeding women who are no more than 6 weeks postpartum, this is based on the time it takes the body to return
to a physiologically nonpregnant state. Thus, by ensuring the breastfeeding participants are in a somewhat similar physiologic state as pregnant participants, potential confounders of hormonal effect on silymarin’s efficacy for melasma treatment will be addressed. To further address potential differences, our study will also use stratified randomization to ensure treatment and control groups have a similar proportion of pregnant to breastfeeding women.

Other exclusion criteria include those with coexisting diseases associated with hyperpigmentation such as thyroid disease or Addison’s disease, those who had melasma development before pregnancy, or those with a known allergy to silymarin\textsuperscript{13,19}. For a full list of inclusion and exclusion criteria, refer to Chapter 3.

2.4.3 Intervention and Method of Administration

The intervention will be topical silymarin cream. Regarding the strength formulation, previous studies have used both 0.7% and 1.4% concentrations\textsuperscript{18,19,22}. One study found no difference between the two concentrations while another study found silymarin’s effect to be dose-dependent with the 1.4% concentration resulting in quicker improvement\textsuperscript{18,19}. Therefore, our study will use 1.4% topical silymarin since no adverse effects were noted with either concentration. It will be prepared with the same formulation noted in previous studies as follows: stearic acid 15 g, glycerin 5 g, KOH 0.72 g, H\textsubscript{2}O 79 g, sodium benzoate (0.1%), Tween-80 (1%), and silymarin 1.4% added. To match for consistency and color, the placebo will be prepared with the same formulation without the silymarin added. Similar to previous trials, participants will be
instructed to apply the cream twice daily to the affected area after cleansing the face to allow for better absorption.

2.4.4 Outcome Measures

The primary outcome of this study will be change in melasma severity from baseline to follow-up at 45 and 90 days. Melasma severity will be measured using the Melasma Area and Severity Index (MASI) score, which is one of the most widely used scoring systems to assess treatment efficacy (cite). This score was developed by Kimbrough-Green et al in 1994 and later validated by Pandya et al in 2011 to be a reliable measure of melasma severity\textsuperscript{30,31}. The MASI score is calculated by assessment of 3 factors: area of involvement, darkness, and homogeneity. Homogeneity and darkness are rated on a 0 to 4 scale (0= absent, 1=slight, 2=mild, 3=marked, 4=maximum). These factors are applied with percentages to 4 areas of the face: the forehead (30%), right malar (30%), left malar (30%), and chin (10%). Despite it being a subjective measure, the study done by Pandya et al. showed MASI score to have good interrater reliability and good validity when compared to more objective measurements such as mexameter readings and computer measurements\textsuperscript{30}. 
The secondary outcome of this study will be change in participants’ quality of life from baseline to the last follow-up at 90 days. This will be assessed using the Melasma Quality of Life scale (MELASQOL) developed by Balkrishnan et al. This disease specific scale was found to be superior to other general measures of health-related quality of life as it placed more emphasis on emotional and psychosocial aspects. MELASQOL has since been used in many studies to evaluate for quality-of-life changes before and after treatment. By using this scale to evaluate for changes in quality of life, our study will be able to gauge patient satisfaction, an important factor in predicting future treatment adherence. No previous study using silymarin for melasma has used this validated scale to assess for patient satisfaction with treatment.

2.4.5 Safety Concerns

Silymarin is not currently FDA approved for any medical use. However, intravenous silibinin, the active component in silymarin, was FDA approved for an
investigational trial for patients with acute hepatotoxicity from mushroom poisoning. From this trial, no serious adverse drug reactions were noted and silibinin administered intravenously was considered safe. Furthermore, despite no FDA approval, silymarin has been used in numerous clinical trials and administered orally, intravenously, and topically. In a review done by Soleimani et al, 43 studies using silymarin reported no adverse reactions. The most common side effect noted was gastrointestinal discomfort including nausea and diarrhea. As these side effects occurred only when silymarin was administered orally, we do not expect the participants in our study to experience GI side effects as we will be using topical silymarin.

As for safety during pregnancy and breastfeeding, silymarin is currently category B with limited studies available on its use during this period. In a study investigating oral silymarin use in pregnant women with chronic hepatitis B, no adverse effects or fetal anomalies were observed. Similarly, a study investigating silymarin’s use as a galactagogue for breastfeeding mothers found no adverse reactions. Furthermore, it was found that total milk production was significantly higher in women taking silymarin, suggesting a possible role for silymarin use in lactation. These studies support the safety of silymarin use in our study, however, it must be noted that little information exists for its use during pregnancy and breastfeeding. For safety reasons, participants in our study will cease treatment if any adverse effects occur.

2.4.6 Sample Size Calculation

The sample size calculation was based on multiple previous studies using the primary outcome of mean MASI score. Studies investigating silymarin for melasma
treatment have used an alpha of 0.05 and power of 80%. Because there are no prior clinical trials examining the effect of silymarin on melasma reduction in a pregnant population, we had to estimate the change in effect size pregnancy would have on treatment. To do so, we used a study by Gharfarzadeh and Eatemadi and a study by Guevara and Pandya to compare the difference in effect size pregnant and non-pregnant populations had on MASI scores of control groups using only sunscreen\textsuperscript{13,40}. From these studies, we estimated the change in effect size would be reduced by 20% in pregnant and breastfeeding women. To estimate the effect size of our intervention, we applied this 20% reduction to a study done by Nofal et al. that found that MASI score was reduced in a non-pregnant silymarin group from 21.75 ± 8.47 to 14.88 ± 7.79\textsuperscript{19}.

Using this data and reduction estimation, for an alpha of 0.05 and a power of 80%, a sample size of 88 was calculated. For additional information, refer to Chapter 3.9.

2.5 Conclusion

In conclusion, our literature review revealed a lack of studies investigating treatment options for melasma during pregnancy. Despite pregnancy being one of the most common triggering factors, only one clinical trial was found that investigated use of a topical agent for melasma treatment in pregnant women\textsuperscript{13}. While most other studies have excluded this population from their trials, this study proved melasma could be successfully treated during pregnancy, prompting the need for other studies targeting this population. Our search also revealed the promising role topical silymarin has in the treatment of melasma. Studies have shown silymarin is as effective as hydroquinone and laser modalities in improving melasma hyperpigmentation but had fewer side effects than
either treatment option\textsuperscript{19,22}. However, these studies are limited in number with smaller sample sizes, indicating the need for further studies on melasma treatment with silymarin.

By investigating the use of silymarin for melasma treatment in a pregnant and breastfeeding population, we aim to address these gaps in the literature with our study. Silymarin reduces the expression of tyrosinase, the enzyme that is stimulated by estrogen and responsible for melanin synthesis\textsuperscript{6,17}. This suggests silymarin may be able to counteract some of the melanogenic effects of increased estrogen levels found during pregnancy. Considering no adverse effects have been reported with topical use, silymarin may be a potential treatment agent in this population that has yet to be explored.
2.6 References


CHAPTER III: STUDY METHODS

3.1 Study Design

This study will be a single-center, double-blind placebo-controlled randomized clinical trial to evaluate the efficacy of topical silymarin for the reduction of melasma severity in pregnant and breastfeeding women. The use of a placebo will help blind the subjects while the investigators will be blinded to the treatment allocation groups. Block randomization will be performed due to use of multiple sites with stratified randomization used to ensure treatment groups contain an equal number of pregnant women and an equal number of breastfeeding women between the two groups.

3.2 Study Population and Sampling

The study population will be women with melasma who are in their second trimester or later of pregnancy or who are currently breastfeeding and no more than 6 weeks postpartum. Those with known thyroid disease, had melasma development before pregnancy, or have a known allergy to silymarin will be excluded from the study. See Table 1 for a complete list of inclusion and exclusion criteria. Eligible participants must be able to provide informed consent and be willing to attend all follow-up visits for the duration of the study. All eligible participants must also have a diagnosis of melasma by a dermatologist before they are able to participate in the trial.

Participants will be chosen from a non-random convenience sample of pregnant and breastfeeding women who meet the inclusion criteria. Recruitment of subjects will occur at Yale outpatient dermatology clinics and at the university’s obstetric centers where women are receiving prenatal care.
Table 1. Inclusion and Exclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Age ≥ 18 years old</td>
<td>• Age &lt; 18 years old</td>
</tr>
<tr>
<td>• Pregnant (confirmed by prenatal ultrasound) or ≤ 6 weeks postpartum</td>
<td>• Male sex</td>
</tr>
<tr>
<td>• No known allergy to silymarin or milk thistle</td>
<td>• Nonpregnant females or females &gt; 6 weeks postpartum</td>
</tr>
<tr>
<td>• Has not received treatment for melasma within the last 6 months</td>
<td>• Known thyroid disorder or other hyperpigmentation disorder (Addison’s)</td>
</tr>
<tr>
<td>• Confirmed diagnosis of melasma</td>
<td>• Known hypersensitivity or allergy to silymarin or milk thistle</td>
</tr>
<tr>
<td>• Melasma development during pregnancy</td>
<td>• Contraindication or sensitivity to sunscreen containing iron oxide</td>
</tr>
<tr>
<td></td>
<td>• Treatment for melasma within the last 6 months</td>
</tr>
<tr>
<td></td>
<td>• Current concomitant use of other skin care products, lasers, or chemical peels</td>
</tr>
<tr>
<td></td>
<td>• Postpartum women taking oral contraceptives</td>
</tr>
<tr>
<td></td>
<td>• Melasma development prior to pregnancy</td>
</tr>
<tr>
<td></td>
<td>• Unavailable for follow-up</td>
</tr>
</tbody>
</table>

### 3.3 Subject Protection and Confidentiality

This study will obtain approval from the Institutional Review Board (IRB) under Yale’s Human Investigations Committee (HIC). All research personnel involved with the study will undergo Health Insurance Portability and Accessibility Act (HIPPA) training and Human Subjects Protection training prior to the start of the trial. Confidentiality of participants will be maintained in accordance with HIPPA. Electronic health records will only be accessed on university-approved encrypted devices and only by HIPPA-trained research personnel involved in direct patient care. Research staff not involved in direct patient care will only have access to de-identified information.
Before initiation of study protocol, all eligible participants must provide written informed consent. The consent form containing the details of the study, risks, benefits, and statements of confidentiality will be explained to each participant individually (APPENDIX A). All subjects will be informed that they may withdraw at any time throughout the study. After the consent form is explained in detail, participants will be given the opportunity to discuss concerns or ask questions before written, signed consent is obtained. Any signed forms or other documents identifying a participant will kept in a locked cabinet. Subjects will be kept informed if significant findings develop during the study that may affect continued participation.

3.4 Recruitment

Subjects will be recruited from outpatient dermatology and obstetrics offices within the Yale Health System. This includes any Yale Health affiliated outpatient offices located in the state of Connecticut. Dermatology and obstetric providers at these clinics will be asked for help in recruitment of eligible patients. Additionally, flyers will be distributed at these sites to help with recruitment and brochures will be provided to potential participants.

Potential participants will be evaluated for eligibility based on the inclusion and exclusion criteria by research personnel. Any participant recruited from an obstetrics office without a diagnosis of melasma will be referred to a dermatologist for evaluation prior to enrollment in the study. Eligible participants will be given the opportunity to enroll and provide informed written consent. Enrolled participants will begin the study while recruitment continues until the target sample size is reached.
3.5 Study Variables and Measures

Participants who have met eligibility requirements will be randomized to one of two groups: the control group or the intervention group. Before receiving any topical treatment, whether it is placebo or silymarin, subjects will be assessed by a licensed dermatology provider to determine baseline characteristics including Fitzpatrick skin type, melasma type, and melasma involved anatomic region. Afterwards, a baseline Melasma Area and Severity Index (MASI) score will be calculated for each subject. All participants will also complete an enrollment survey and the Melasma Quality of Life questionnaire at baseline. The enrollment survey will ask participants about age, parity, gravidity, estimated sun exposure per day, and family history. Any missing demographic information not filled out on the survey will be obtained through the EHR, if possible.

The intervention group will receive a 45-day supply of topical 1.4% silymarin cream in an unmarked pump bottle that will be replaced at every follow-up visit until the end of the study. The control group will receive an identical unmarked pump bottle containing a 45-day supply of placebo cream. To ensure similar consistency and color, the placebo cream will be prepared using the same formula as the silymarin cream without the silymarin added. Participants in both groups will be instructed to apply one pump of cream twice daily, once in the morning and once before bedtime, after thoroughly cleansing and drying the face. Participants must also agree to not use any other topical treatments for the duration of the study. Adherence to treatment can be evaluated at every follow-up visit using the 45-day supply bottle as excessive leftover cream would indicate non-adherence.
To ensure adequate sun protection, all participants will receive SPF 50 sunscreen and told to avoid excessive sun exposure. They will be instructed to apply the sunscreen daily after applying the treatment cream and to reapply the sunscreen every 2 hours when spending time outdoors.

The primary outcome of this study will be change in melasma severity score assessed using the Melasma Area Severity Index (MASI). A baseline MASI score will be calculated by a trained research assistant at the time of enrollment with additional MASI scores calculated at every follow-up visit. To effectively analyze therapeutic response, mean MASI scores will be operationalized as a continuous variable to compare scores throughout the treatment course\textsuperscript{1-3}. Since melasma is known to affect quality of life, a secondary outcome of this study will evaluate changes in the participants’ quality of life. Change in quality of life will be assessed at baseline and at the end of the study using the Melasma Quality of Life (MELASQOL) questionnaire. Additionally, any adverse effects of treatment will be evaluated and recorded at each follow-up visit.

3.6 Blinding of Intervention and Outcome

By using a placebo that is matched in appearance to the intervention cream, participants will be blinded to intervention assignment. All participants will also be provided with the same instructions for application regardless of group assignment. The silymarin and placebo formulations will be prepared at one clinical center then distributed to each site to ensure investigators and research assistants at each site are blinded to treatment allocation when distributing the topical creams. These blinded investigators and research assistants will be the study personnel responsible for outcome assessment.
including calculation of MASI score at each follow-up visit. Participants and outcome assessors will not be told the hypothesis of the study until the study has ended.

3.7 Assignment of Intervention

Because multiple study locations are used, block randomization will be utilized at each study site to ensure equal allocation into treatment groups. After enrollment, participants will complete baseline assessments then be stratified by pregnant or postpartum status before randomization to intervention. This stratified randomization will help to ensure equal distribution of pregnant and breastfeeding women into the intervention or control group. After stratification, randomization of participants will be performed by a computerized program in a 1:1 ratio of intervention to control group. A unique study identification number will be assigned to each subject at this time.

3.8 Data Collection

After informed consent is obtained to enroll in the study, all participants will undergo an assessment by a licensed dermatology provider to obtain baseline information on Fitzpatrick skin type, melasma type, and melasma involved anatomic region. Participants will also complete a baseline characteristics survey for demographic information. Research staff trained in MASI scoring will calculate and record a baseline MASI score for each subject prior to beginning treatment (refer to Chapter 2 Figure 1 for MASI formula). As the primary outcome of the study, MASI score will be calculated and recorded at each follow-up visit by blinded research staff.
The secondary outcome of quality-of-life changes and patient satisfaction with treatment will be measured by administering the MELASQOL questionnaire (APPENDIX B) at baseline and at the last follow-up visit at day 90 of treatment. The MELASQOL requires subjects to rate on a scale of 1 to 7 how they feel about a series of ten statements. Scores range from 7 to 70 with higher scores indicating a poorer quality of life. Participant’s answers will be recorded for each statement and totaled at the end.

Adherence will be monitored at every follow-up visit as subjects will be asked to bring their used topical formulation bottle to exchange for a new 45-day supply bottle. Any large excess of remaining formula in used bottles will prompt a discussion with the participant that may lead to recording that subject as nonadherent to treatment. Lastly, any adverse effects to treatment will be monitored and noted throughout the duration of the study.

3.9 Sample Size Calculation

Using the Power and Precision 4 software for a two-tailed test and an alpha of 0.05, a sample size of 88 participants was determined. As discussed in Chapter II, the sample size calculation was based on multiple previous studies using the primary outcome of mean MASI score. From these studies, an estimated 20% reduction in effect size due to using a pregnant and postpartum population was applied to the effect size found in a non-pregnant sample by Nofal et al.\(^3\). Thus, the sample size of 88 was calculated. Additionally, we will account for a 10% lost to follow-up which brings our final sample size to 98 participants, or 49 participants in each group. (See APPENDIX C for Sample Size Calculation)
3.10 Analysis

Demographic information of the participants will be collected at baseline and analyzed for any statistically significant between group differences. Characteristics such as age, Fitzpatrick skin type, parity, gravidity, melasma type, melasma involved anatomic region, estimated sun exposure, and family history will be analyzed. Student t-test will be used to analyze any continuous, parametric variables such as age and estimated sun exposure. Number and percentage will be used for categorical variables such as Fitzpatrick skin type, melasma type, melasma involved anatomic region, and family history. These categorical variables will be analyzed using a chi-square test. See Table 2 for more information.

Table 2. Baseline Characteristics, Description, and Analysis

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>Description</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean age (yrs) ± SD</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Fitzpatrick skin type</td>
<td>I,II,III,IV (No./total No. (%))</td>
<td>Chi-square test</td>
</tr>
<tr>
<td>Parity</td>
<td>No./total No. (%)</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Gravidity</td>
<td>No./total No. (%)</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Melasma type</td>
<td>Dermal, epidermal, mixed (No./total No. (%))</td>
<td>Chi-square test</td>
</tr>
<tr>
<td>Melasma anatomic region</td>
<td>Centrofacial, malar, mandibular (No./total No. (%))</td>
<td>Chi-square test</td>
</tr>
<tr>
<td>Estimated sun exposure per day</td>
<td>Mean time (hrs) ± SD</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Family History</td>
<td>Yes, No (No./total No. (%))</td>
<td>Student t-test</td>
</tr>
</tbody>
</table>

The primary analysis will be an intention-to-treat analysis. Results will be considered statistically significant for p values <0.05. The primary outcome of change in melasma severity from baseline will be evaluated as a continuous variable using mean MASI score. Therefore, mean MASI score will be analyzed using paired t-test. If MASI scores are not normally distributed, Wilcoxon signed-rank test will be used. The secondary outcome of change in mean MELASQOL score from baseline will be
evaluated as a continuous variable and analyzed using paired t-test. Any adverse effects will be categorized and analyzed using chi square test.

### 3.11 Timeline and Resources

The proposed study including recruitment, randomization, and data collection will take course over a two-year span. The first 3 months of the study will be used to coordinate with clinical sites and train research staff on MASI scoring as well as other assessment tools. The following 18 months will be used for rolling recruitment and enrollment of eligible subjects.

All study participants will meet with a dermatology provider and research assistant within the first week of enrollment for baseline assessments and to receive their topical treatment. The total duration of treatment will be 90 days with follow-up visits conducted by research assistants at day 45 of treatment and day 90. The last 3 months of the study will be used to for data analysis and to continue data collection on subjects enrolled in the last month of recruitment. See Figure 2 for the proposed study timeline.

**Figure 2. Study Timeline**

The principal investigator (PI) for the study will be Dr. Suguru Imaeda and the co-principal investigator will be Warunee Nowak, PA-SII. Research assistants will be
needed at each clinical site to obtain informed consent, administer baseline surveys, and provide enrolled participants with instructions on adherence and follow-up. These assistants will also be responsible for recording MASI score calculations at baseline and follow-up visits. Participants will be compensated for their time at both follow-up visits with a $15 Visa gift card.
3.12 References


CHAPTER IV: CONCLUSION

4.1 Advantages and Disadvantages

The proposed study has several advantages. It will be the first randomized clinical trial to evaluate the efficacy of topical silymarin in treating melasma in pregnant and breastfeeding women. Furthermore, it will only be one of two studies to investigate a non-sunscreen melasma treatment agent for use during pregnancy\(^1\). Other studies have shown silymarin is effective at reducing MASI score and improving hyperpigmentation, but these studies had small sample sizes and signs of bias\(^2-4\). Our study hopes to improve on these limitations by enrolling a larger sample size of 98 participants to increase generalizability. Additionally, by sampling from multiple sites and by recruiting from obstetrics offices as well as dermatology clinics, we aim to ensure our results are more generalizable to the pregnant and postpartum population. Another advantage of our study is its double-blind design which allows us to limit potential sources of bias. Other silymarin studies either did not blind participants or investigators, or they utilized a single-blind design due to the impossibility of blinding participants to the comparison group being used\(^2,3\). This jeopardizes the validity of results as both MASI and patient satisfaction scores used in these studies are subjective measures. Our study addresses this issue by using placebo to blind participants and using investigators who are blinded to intervention allocation. Lastly, our study will be the first silymarin trial to evaluate quality of life changes in response to treatment using the MELASQOL questionnaire. Given the detrimental effects melasma has on psychological and social wellbeing, it is important to assess silymarin’s effect on this aspect of patient’s lives\(^5,6\).
Despite the advantages of our study, there are a few limitations that need to be considered. First, our study uses subjective measures for both our primary and secondary outcomes. Our primary outcome of MASI score is a subjective measure which can contribute to bias or differences with calculation across the multiple sites in our study. We attempt to reduce this source of bias in MASI scoring by blinding the assessors and by providing all sites with standard instructions and training on MASI calculation. MASI score has been validated and shown to have good interrater reliability\(^7\). As for our secondary outcome, blinding of the participants will attempt to negate any participant bias when answering the MELASQOL questionnaire. Another limitation is our use of convenience sampling which can limit the generalizability of our results. We aim to reduce some of the disadvantages of convenience sampling by recruiting from obstetrics offices in addition to dermatology offices. This allows us to increase the heterogeneity of our sample to ensure individuals who are not likely to present to a dermatology office are included in our study. In summary, although several limitations exist in our study, measures were incorporated in our study’s design to lessen the effects of these limitations on our study’s validity.

### 4.2 Clinical Significance

Melasma negatively impacts the lives of affected patients with adverse effects on social and psychological wellbeing\(^8\). Because the hyperpigmentation patches are highly visible, melasma can lead to feelings of frustration, embarrassment, and even depression\(^9\). Furthermore, there seems to be no association between clinical severity and effects on quality of life, indicating a need for treatment even in mild cases to minimize its negative
The results of our study could support the use of a new melasma treatment agent: topical silymarin. Because very limited data exists on the efficacy of topical silymarin, there is no established role for its use clinically in the treatment of melasma. However, our study could add to the existing literature to support silymarin’s efficacy in treating these hyperpigmentation patches.

More importantly, our study targets a population with no current first-line treatment options. Considering the detrimental effects on quality of life, melasma should be treated as it develops, but limited options exist for pregnant and breastfeeding women. Current standard of care during this period focuses exclusively on preventive measures such as sunscreen use and sun avoidance. If this study can show the success of silymarin in treating pregnancy-induced melasma, providers may have a new alternative to offer pregnant and lactating women looking for treatment. The results of our study would provide clinicians with data to make informed decisions and recommendations regarding the use of silymarin for melasma treatment during pregnancy and the postpartum period.
4.3 References

APPENDICES

APPENDIX A: AUTHORIZATION AND CONSENT FORM

COMPOUND AUTHORIZATION AND CONSENT FOR PARTICIPATION IN A RESEARCH STUDY

YALE UNIVERSITY SCHOOL OF MEDICINE

Study Title: Efficacy of Topical Silymarin in Melasma Treatment During Pregnancy and Breastfeeding

Principal Investigator: Suguru Imaeda, MD
Co-Investigator: Warunee Nowak, PA-SII

Research Study Summary:

- We are asking you to join a research study.
- The purpose of this research study is to investigate the efficacy of topical silymarin in treating melasma during pregnancy and breastfeeding.
- Study procedures will include: Twice daily application of placebo or silymarin cream (randomly assigned), along with application of SPF 50+ sunscreen daily. Two follow-up visits to record progress with treatment and administer follow-up questionnaire.
- 3 visits are required. One around the time of enrollment, then another 45 and 90 days after beginning treatment.
- These visits will take 6 hours total.
- There are some risks from participating in this study. Potential risks include skin irritation, hypersensitivity reaction, and/or a breach of confidentiality. However, these risks are very unlikely.
- The study may or may not have any benefit to you. The benefits to science and other people may include a better understanding of using topical silymarin in the treatment of melasma during pregnancy and breastfeeding.
- There are other choices available to you outside of this research. Current standard of care for melasma during this period focuses on prevention through use of sunscreen and sun avoidance. We will ask you to do both as part of our study as well should you agree to participate.
- Taking part in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You can also change your mind at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.
- If you are interested in learning more about the study, please continue reading, or have someone read to you, the rest of this document. Take as much time as you need before you make your decision. Ask the study staff questions about anything you do not understand. Once you understand the study, we will ask you if you wish to participate; if so, you will have to sign this form.
**Why is this study being offered to me?**
We are asking you to take part in a research study because you are a second trimester or later pregnant or no more than 6 weeks postpartum individual with melasma who is interested in treatment. We are looking for 98 pregnant or breastfeeding participants to be part of this research study.

**Who is paying for the study?**

**Who is providing other support for the study?**
Yale University School of Medicine Physician Associate Program

**What is the study about?**
The purpose of this study is to test how well 1.4% topical silymarin works for the treatment of melasma in pregnant and breastfeeding women. Topical silymarin is not currently FDA approved for treatment. However, it has been used in other research trials with success. It is currently Category B for pregnancy indicating it is considered safe to use during pregnancy.

**What are you asking me to do and how long will it take?**
If you agree to take part in this study, you will randomly be assigned by a computer to either (a) the control group, which will receive a placebo cream OR (b) the intervention group, which will receive silymarin cream. Both groups will receive SPF 50+ sunscreen.

You will be instructed to apply your cream twice daily. Once in the morning after cleansing and drying the face, you will apply one pump of cream to affected melasma areas of your face. After the cream, you will apply ¼ teaspoon of SPF 50+ sunscreen all over your face. If you are outdoors for an extended period, you will need to reapply the sunscreen every 2 hours. The second application of the cream will be before bedtime. Again, one pump of cream will be applied to affected areas after cleansing and drying the face. You will need to continue this routine until the end of the study at 90 days. By agreeing to participate in this study you agree to not use any other treatments, topical or otherwise, for the duration of the study.

During the study there will be 3 total visits you will need to attend. The first visit will occur shortly after enrollment. At this visit, a dermatology provider will assess your skin to gather some baseline information on your melasma. A research assistant will also be present to administer two surveys. One will be for information regarding your age, family history, how many times you’ve been pregnant, estimated sun exposure, etc. The other will be to assess your quality of life using a validated questionnaire. At this visit, you will also receive your topical cream.

For the next 2 visits, you will report to the same as your initial visit on day 45 and day 90 of using the topical cream. On the day 45 visit, you will meet with a research assistant
who will record your progress with the treatment and who will replenish your treatment supply and sunscreen supply. On the day 90 visit, you will meet with a research assistant who will record your final melasma score. You will also be asked to take the same quality of life survey you took at enrollment.

**What are the risks and discomforts of participating?**

We do not anticipate any major risks or discomforts of participating in this study. 1.4% topical silymarin has been studied in several clinical trials without any adverse effects reported. However, limited information is available on its use during pregnancy. Potential risks include skin irritation or hypersensitivity reaction, although these are highly unlikely. There is a risk of breach of confidentiality about your health status and participation in this study, though this is unlikely to occur. All research staff will be thoroughly trained and certified in the privacy of research studies.

**How will I know about new risks or important information about the study?**

We will tell you if we learn any new information that could change your mind about taking part in this study.

**How can the study possibly benefit me?**

Benefits of participation in this study may include improvements in skin hyperpigmentation which may lead to improvements in quality of life. However, this is not guaranteed, and you may not benefit directly from your participation.

**How can the study possibly benefit other people?**

The benefits to science and other people may include a better understanding of use of topical silymarin in the treatment of melasma. This includes more information about its effectiveness during pregnancy and breastfeeding.

**Are there any costs to participation?**

If you take part in this study, you will not have to pay for any services, supplies, study procedures, or care that are provided for this research only (they are NOT part of your routine medical care). However, there may be additional costs to you. These can include costs of transportation and your time to come to the study visits. You or your health insurance must pay for services, supplies, procedures, and care that are part of your routine medical care. You will be responsible for any co-payments required by your insurance. You will not have to pay for taking part in this study. The only costs include transportation and your time coming to the study visits.

Your study doctor, a member of the study team, or a member of the research billing team will be glad to answer your questions about whether services or tests performed during
the course of a research study will be billed to your insurance provider or to you, or about any bills that you may receive during your participation in a research study. Please call the research billing team at 1-877-TRIALS0 (1-877-874-2560) with any questions. You may also contact your insurance provider directly.

**Will I be paid for participation?**

You will be paid for taking part in this study. You will be given a $15 Visa gift card at each follow-up visit at day 45 and day 90 of the study. If you do not come to these visits, you will not be eligible to receive the gift card. You are responsible for paying state, federal, or other taxes for the payments you receive for being in this study. Taxes are not withheld from your payments.

**What are my choices if I decide not to take part in this study?**

Instead of participating in this study, you have some other choices. You could:

- Get treatment without being in a study. However, this treatment is not currently available without participation. Current treatment options for melasma during pregnancy focus on using sunscreen for prevention, which will also be provided in this study.
- Take part in another study.
- Receive no treatment for your disease. Melasma is a benign condition and treatment is not necessary.

**How will you keep my data safe and private?**

We will keep information we collect about you confidential. We will share it with others if you agree to it or when we have to do it because U.S. or State law requires it. For example, we will tell somebody if you we learn that you are hurting a child or an older person.

Information will be kept confidential by using your study identification number on study forms, storing signed forms in locked cabinets, and storing any research data on password-protected computers. Any information that identifies your personal health information will be deidentified before distribution among members of the research team.

When we publish the results of the research or talk about it in conferences, we will not use your name. If we want to use your name, we would ask you for your permission.

We will also share information about you with other researchers for future research but we will not use your name or other identifiers. Identifiers will be removed from identifiable private information, and after removal, the information could be used for future research studies or distributed to another investigator for future research studies. We will not ask you for any additional permission.

**What Information Will You Collect About Me in this Study?**
The information we are asking to use and share is called “Protected Health Information.” It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). In general, we cannot use or share your health information for research without your permission. If you want, we can give you more information about the Privacy Rule. Also, if you have any questions about the Privacy Rule and your rights, you can speak to Yale Privacy Officer at 203-432-5919.

The specific information about you and your health that we will collect, use, and share includes:

- Research study records
- Medical and laboratory records of only those services provided in connection with this Study.
- The entire research record and any medical records held by Yale Health
- Records about phone calls made as part of this research
- Records about your study visits
- Information obtained during this research regarding
  - Physical exams
  - Diaries and questionnaires
  - Records about any study drug you received

**How will you use and share my information?**

We will use your information to conduct the study described in this consent form. We may share your information with:

- The U.S. Department of Health and Human Services (DHHS) agencies
- Representatives from Yale University, the Yale Human Research Protection Program and the Institutional Review Board (the committee that reviews, approves, and monitors research on human participants), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- The U.S. Food and Drug Administration (FDA) This is done so that the FDA can review information about 1.4% topical silymarin involved in this research. The information may also be used to meet the reporting requirements of drug regulatory agencies.
- The study sponsor or manufacturer of study drug/device
- Drug regulatory agencies in other countries
- Governmental agencies to whom certain diseases (reportable diseases) must be reported
- Health care providers who provide services to you in connection with this study.
- Laboratories and other individuals and organizations that analyze your health information in connection with this study, according to the study plan.
- Principal Investigator of the study
- Co-Investigators and other investigators
- Study Coordinator and Members of the Research Team
- Data and Safety Monitoring Boards and others authorized to monitor the conduct of the Study
We will do our best to make sure your information stays private. But, if we share information with people who do not have to follow the Privacy Rule, your information will no longer be protected by the Privacy Rule. Let us know if you have questions about this. However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

**Why must I sign this document?**
By signing this form, you will allow researchers to use and disclose your information described above for this research study. This is to ensure that the information related to this research is available to all parties who may need it for research purposes. You always have the right to review and copy your health information in your medical record.

However, this is a double blinded treatment study and if you sign this permission form, you will not be allowed to look at or copy you study related information until after the research is complete.

**What if I change my mind?**
The authorization to use and disclose your health information collected during your participation in this study will never expire. However, you may withdraw or take away your permission at any time. You may withdraw your permission by telling the study staff or by writing to Suguru Imaeda at the Yale University, New Haven, CT 06520.

If you withdraw your permission, you will not be able to stay in this study but the care you get from your doctor outside this study will not change. No new health information identifying you will be gathered after the date you withdraw. Information that has already been collected may still be used and given to others until the end of the research study to ensure the integrity of the study and/or study oversight.

**What if I want to refuse or end participation before the study is over?**
Taking part in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You also can change your mind at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.

We would still treat you with standard therapy or, at your request, refer you to a clinic or doctor who can offer this treatment. Not participating or withdrawing later will not harm your relationship with your own doctors or with this institution.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part.
The researchers may withdraw you from participating in the research if necessary. This may be due to development of serious side effects or you no longer meet the inclusion criteria.

**What will happen with my data if I stop participating?**

You may withdraw or take away permission to use and disclose your health information at any time. When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to ensure the integrity of the study and/or study oversight.

**Who should I contact if I have questions?**

Please feel free to ask about anything you don't understand.

If you have questions later or if you have a research-related problem, you can contact the Co-Principal Investigator at warunee.nowak@yale.edu

If you have questions about your rights as a research participant, or you have complaints about this research, you call the Yale Institutional Review Boards at (203) 785-4688 or email hrpp@yale.edu.

A description of this clinical trial will be available on [http://www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

**Authorization and Permission**

Your signature below indicates that you have read this consent document and that you agree to be in this study.

We will give you a copy of this form.

<table>
<thead>
<tr>
<th>Participant Printed Name</th>
<th>Participant Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Obtaining Consent Printed Name</td>
<td>Person Obtaining Consent Signature</td>
<td>Date</td>
</tr>
</tbody>
</table>
APPENDIX B: SAMPLE MELASQOL

The Melasma Quality of Life Questionnaire

Instructions: On a scale of 1 (not bothered at all) to 7 (bothered all the time), please rate how you feel about the following:

(____) The appearance of your skin condition

(____) Frustration about your skin condition

(____) Embarrassment about your skin condition

(____) Feeling depressed about your skin condition

(____) The effects of your skin condition on interactions with other people (e.g., interactions with family, friends, and close relationships)

(____) The effects of your skin condition on your desire to be with people

(____) Your skin condition makes it hard to show affection

(____) Skin discoloration makes you feel unattractive to others

(____) Skin discoloration makes you feel less vital or productive

(____) Skin discoloration affects your sense of freedom

Total score added up: __________

The MELASQOL is scored from 7 to 70.
### APPENDIX C: SAMPLE SIZE CALCULATION

<table>
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<tr>
<th>Group</th>
<th>Population Mean</th>
<th>Standard Deviation</th>
<th>N Per Group</th>
<th>Standard Error</th>
<th>95% Lower</th>
<th>95% Upper</th>
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</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>54.3</td>
<td>8.4</td>
<td>44</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>13.2</td>
<td>2.7</td>
<td>44</td>
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</tr>
</tbody>
</table>

Mean Difference: 30.2

Alpha = 0.001, Table 2

**Summary - Power**

For the given effect size (population means of 54 vs 13.6), 50 (6.4 vs 27), sample sizes (44 and 44), and alpha=0.05 (2-sided), power is 0.996.

This means that 99% of studies would be expected to yield a significant effect, rejecting the null hypothesis that the two population means are equal.

Power and Precision Version 4.0 Biostat Inc., Englewood, NJ
BIBLIOGRAPHY


