Evaluation of the galactogogue effect of silymarin on mothers of preterm newborns (<32 weeks)

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Abstract

Hypogalactia has a relative high frequency in women having delivered preterm infants, who often have difficulties in maintaining a sufficient production of milk for their infants' needs over prolonged periods of time. Recent studies have shown a potential galactogogue effect of silymarin on milk production in animal models (cows and rats) and in humans (mothers of term newborns); nonetheless, none of the studies conducted on humans consisted of double-blind randomized clinical trials and no data are available concerning mothers who delivered preterm infants. The aim of our study was to assess the efficacy of silymarin (BIO-C®) as galactogogue and its tolerability in mothers who delivered preterm infants. We enrolled 50 mothers at 10±1 days *post-partum* who had delivered infants at <32 weeks' gestation. They were randomly assigned to receive sachets indistinguishable for taste and formulation, containing silymarin or placebo. The mothers were asked to write down the amount of milk they had produced for each extraction using a breast pump or weighing the baby before and after the breastfeeding. No differences emerged in milk production profile between the silymarin BIO-C® and placebo arms. No adverse events were observed in the 2 arms among mothers and infants, and silymarin and its metabolites were not detectable in the analyzed human milk samples. Further investigation on specific patient groups affected by hypogalactia, defined according to stricter criteria, should be planned to assess the efficacy of the product in increasing milk production.

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Introduction

Breast milk is currently considered to be the optimal form of enteral nutrition for term and preterm infants until up to six months postnatal age.[1]

Mothers of preterm neonates have difficulty in maintaining sufficient volume of human milk for their infants' needs over prolonged periods of time.[2] Anxiety, fatigue and emotional stress, illness of the mother or of the baby, incorrect breast attachment or inadequate breast milk emptying during manual or mechanical extraction[3,4] could be inhibitors of lactation, despite appropriate lactation counseling and the use of non-pharmacologic strategies.

When other non-pharmacological measures do not produce an increase in milk volume, galactogogues can be recommended. Galactogogues are medications or other substances believed to assist initiation, maintenance or augmentation of maternal milk production.[5]

Domperidone is a medication used like galactogogue and two studies suggest modest improvements in short-term expressed breastmilk volumes when a medication is used after insufficient expressed breastmilk occurs in mothers following preterm delivery.[6,7] A possible alternative can be represented by natural products. The mechanisms of action for most herbals are unknown and most of them have not been scientifically evaluated.[5] Some herbs mentioned as galactogogues include fenugreek, galega and milk thistle (*Silybum marianum*).[8]

Silymarin is an extract, characterized by the presence of a flavonolignans fraction (a mixture of silybin, silycristine and silydianin), obtained from *Silybum marianum*.[4] This is a well-known medical plant whose standardized extract is often used in phytotherapy and in allopathic medicine mainly as a liver protective agent.[9]

Studies have shown a potential galactogogue effect of silymarin in milk production in animal models (cows and rats).[10-12] Di Pierro conducted a single-blind randomized clinical trial, and demonstrated that silymarin BIO-C® significantly increases milk produced by

mothers of term newborns. This study stated a good tolerability of the product as well as lack of passage in women's milk.[13]

The aim of our study was to assess silymarin BIO-C® efficacy as galactogogue, its tolerability and its pharmacodynamics in mothers who delivered preterm babies, by a double-blind randomized clinical trial.

Materials and Methods

Setting and population

This study was conducted at the Neonatal Intensive Care Unit of the Department of Public Health and Pediatric, University of Turin, Italy between 2011 and 2012. The protocol of the study was approved by the Ethics Committee of the Italian Association of Human Milk Banks (AIBLUD). The target population consisted of women who had delivered infants at <32 weeks' gestation and gave written informed consent to participate in the study. Women were considered ineligible if smokers and if they could not breastfeed because of their clinical conditions, such as HIV infection.

Study design

Eligible and consenting women were randomly assigned by using a computer-based code. The manufacturer of BIO-C® prepared two boxes of sachets indistinguishable for taste and formulation; one of this contained silymarin in the form of BIO-C® (corresponding to a daily dose: *Cardus Marianus* L. dry extract 420 mg, micronized silymarin 252 mg), the other one a placebo (maltodextrin). Before packaging, the active drug was micronized, a procedure that improves its oral bioavailability. Mothers were enrolled in the study 10±1 days *post-partum*. The posology was one sachet every 12 hours for 28 days; the dose was decided on the basis of the current clinical practice.

After randomization, participants received their drug or placebo supply for the first 7 days, together with instructions for self-administration. The sachets were supplied to the mothers every 7 days (for a total 28 days) to check the proper intake and to evaluate the compliance to the study protocol.

Baseline measurements were taken and recorded after randomization (day 0). Mothers were asked to express their milk according to the standard neonatal intensive care unit (NICU) practice: they extracted their milk at least 6 times per day by Medela Simphony breast pump (given to the mothers after the discharge; Medela Inc., McHenry, IL, USA); they stopped the milk extraction one minute after the leaking of the last drop of milk or, if there was no milk, after 5-10 minutes. If the baby could suck, the amount of milk was measured weighing the baby before and after the breastfeeding using an electronic scale, given to the mother after the discharge (precision ±1 g, mod. Baby One; La Precisa, Turin, Italy). Qualified staff supported each mother in breastfeeding, giving them written recommendation and practical support, focusing on frequency and duration of the extraction, on the use of the electric breast pump (not used on both breast simultaneously) and giving advice on diet.

Dedicated recording forms were given to the mothers, who had to write down the amount of milk they had produced for each extraction, from both breasts, using the breast pump or weighing the baby before and after the breastfeeding. In both arms daily milk volumes were calculated at day 0 (that is, the amount of milk produced in the 24 hours before the start of administration of the sachets), every day in the first week and at study days 14, 21, 26, 27 and 28 (end of treatment).

The volume of milk extracted was also recorded at day 36 and 43, whereupon it was also verified that mothers did not take any medication to increase lactation.

To evaluate the excretion of silymarin in human milk, a milk sample for each mother was analyzed at study day 14 by an external laboratory.

Mothers were also asked to record any personal feedback about the substance they were taking at study day 7, 14, 21 and 28 and to report any side effect.

Aim and study end-points

The purpose of the study was to assess the silymarin galactogogue action in human milk production. The primary endpoint was the difference between the mean milk production after 26, 27 and 28 days of administration and basal production. The secondary endpoints included: i) the variation in milk production with respect to the baseline, after 1, 2, 3, 4, 5, 6, 7, 14, 21 days of administration; ii) the variation in milk production with respect to the baseline after 8 and 15 days after the end of administration (36th and 43th day, respectively); iii) occurrence of side effects; iv) silymarin excretion in human milk; v) subjective perception of substance efficacy.

Study size

According to Fewtrell and colleagues,[9] the standard deviation (SD) of the mean milk production in the second week after a preterm delivery is about 160 g/day, whereas is about 280 g/day after one month since a term delivery. We considered a 150 g/day difference in milk production variation between silymarin and placebo, after 28 days of treatment, as the minimum clinically important effect. If we set to α=0.05 (one-tail test) the type I error risk and to (1-β)=0.80 the power of the study, and assume that the variation in milk production has SD=200 g/day, we find that the required study size is 23 subjects per arm. In the period February 2011-October 2012 we enrolled 50 women who were randomized 1:1 to silymarin or placebo.

Statistical methods

As for the primary endpoint, Student's t test for independent samples was used to test the between-treatment difference of variation in milk production between day 0 and days from 26 to 28.

As for secondary endpoints, daily milk production profile was fitted with a mixed linear model including arm (silymarin or placebo), subject within arm, time and interaction arm×time as covariates. Assumption of 1st order autoregressive heterogeneous covariance matrix between the observations of response profile was made. The linear and quadratic components of milk production profile and their interactions with arm were evaluated.

To investigate the subjective perception of substance efficacy, women were divided in two groups on the basis of their guess on the 28th day about the substance they were taking: the group of silymarin-guess and the group of placebo-guess. Student's t test for independent samples was used to test, between guess groups, the difference of variation in milk production between day 0 and days from 26 to 28.

The analyses were carried out using PROC TTEST, GLM, SGPLOT and MIXED of SAS software, version 9.4 of the SAS System (SAS Institute Inc., Cary, NC, USA).

Silymarin excretion in human milk: analytical method

Milk samples were extracted in methanol in an ultrasonic bath. The solution was then centrifuged, filtered at 0.45 mm and analysed by high performance liquid chromatography coupled to an ultraviolet-visible spectrophotometry detector and using adequate silymarin standard solutions as analytical control. The total silymarin content in the sample (expressed as percentage) is computed by comparison with the analytical control adding the peaks corresponding to the identified silymarin metabolites (silychristin, silydianin, silibyn A, silibyn B, isosilybin A and isosilybin B).

Results

Maternal and infant characteristics at baseline in the silymarin and placebo arms are shown in Tables 1 and 2. The frequency of multiparae was higher in sylimarin arm, as well as the duration of breastfeeding in women that had breastfed previously.

Two women in the silymarin arm withdrew from the study at day 4 and 7 for use of formula milk and intercurrent disease respectively, and two women in the placebo arm at day 14 for critical condition of neonates.

Maternal age, hypertension, parity, multiple pregnancy, diabetes and gestational age showed no important effect on the primary endpoint (P>0.3 for all effects).

Figure 1 shows average milk production and 95% confidence intervals in the two arms from baseline to the 43rd day. The higher milk production observed in the silymarin arm at the baseline (day 0) persisted up to the 43rd day. Negligible differences in milk production variation between baseline and the mean of days 26-28 (primary endpoint) was observed between the two arms (sylimarin: 24.9±38.1 g/day; placebo: 77.3±24.9; difference sylimarin-placebo: -52.4±45.5 g/day, P=0.872).

Figure 2 shows average milk production profiles with their 95% confidence intervals (CI) estimated with the mixed linear model. No difference in milk production profile emerges between silymarin and placebo arms, as indicated by the lack of interaction arm×time in its linear and quadratic components (P=0.332 and P=0.254, respectively). Both arms show a sharp irregular variation in milk production in the first week after enrollment followed by a slow decrease (quadratic component: P<0.001).

On day 28th, 17 women believed they were taking silymarin (silymarin-guess group: 8 of silymarin-arm, 7 of placebo-arm), 28 women believed they were taking placebo (placebo-guess group: 14 of silymarin

Table 1. Maternal characteristics at randomization.

	Arm	
	Silymarin(n=25)	Placebo(n=25)
Median age (year)	35 (24-42)	33 (26-56)
Italian nationality (n)	23	20
Qualification (n)		
Middle school	4	3
High school	16	14
University degree	5	8
Diabetes (n)	1	2
Hypertension (n)	9	13
Other chronic disease (n)	9	3
Multiparae (n)	10	5
Months breastfeeding at last pregnancy (mean±SE g/day)	11.8±2.3	6.6±1.8
Cesarean delivery (n)	19	19
Drugs in pregnancy (n)	24	25

SE, standard error. None of the 50 women had immunization. Values in parenthesis represent range.

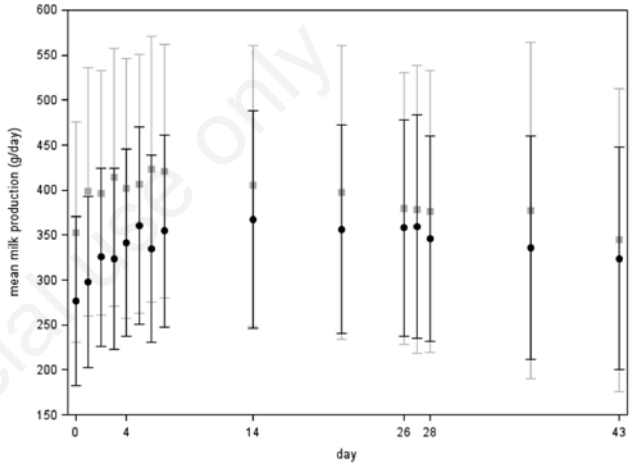

Figure 1. Average milk production (g/day) and 95% confidence intervals in silymarin (grey squares) and placebo (black dots) arm.

Table 2. Infant characteristics at randomization.

	Arm	
	Silymarin(n=31)	Placebo(n=31)
Median gestational age (week)	29 (24-31)	29 (25-31)
Birth weight (mean±SE), g	1105±67	1050±50
Twins (n)	12	12
FGR (n)	12	13
Gender, male (n)	17	19
RDS (n)	30	29
PDA (n)	14	7
IVH > grade 2 (n)	1	1
Feeding intolerance (n)	6	5

SE, standard error; FGR, fetal growth restriction; RDS, respiratory distress syndrome; PDA, patent ductus arteriosus; IVH, intraventricular hemorrhage. None of the 62 neonates had necrotising enterocolitis. Values in parenthesis represent range.

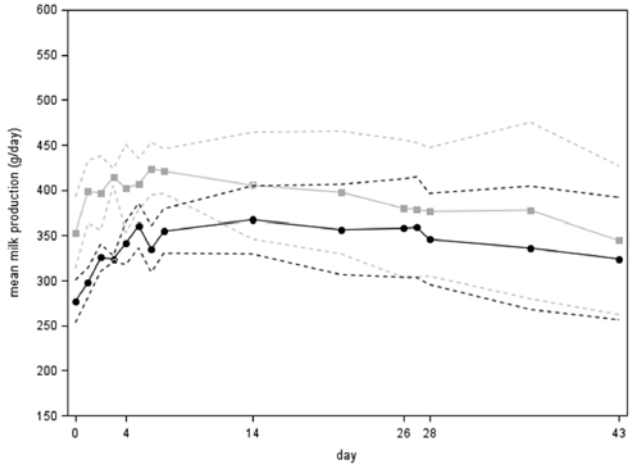

Figure 2. Average profile of milk production (g/day) and 95% confidence intervals in silymarin (grey squares) and placebo (black dots) arm. Individual profiles were fitted with a mixed linear model.

arm, 14 of placebo arm), whereas one did not say her guess. Figure 3 shows average milk production and 95% CI of milk production in the two-guess groups from baseline to the 43rd day. As expected, women who believe they were taking silymarin were those with higher milk production, but this difference was observed already at baseline. Negligible differences in milk production variation between baseline and the mean of days 26-28 was observed between the two guess-groups [silymarin-guess: 61.6±35.7 g/day; placebo-guess: 30.9±19.6 g/day; difference (silymarin-guess)-(placebo-guess): +30.7±48.4 g/day, P=0.529].

No important adverse events were reported for both mothers and infants.

Silymarin concentration in human milk was not detectable (<0.3 mg/kg) at day 14 in any of the samples analyzed.

Discussion

Neonatal intensive care unit is a common area of use of the galactogogues: the aim is to stimulate initial secretory activation or reduce the decline of milk secretion in these mothers. Prior to the use of a galactogogue, the entire feeding process should be thoroughly evaluated by a lactation expert. Reassurance may be offered, if appropriate. When intervention is indicated for the dyad, modifiable factors should be addressed: comfort and relaxation for the mother, frequency and thoroughness of milk removal, and underlying medical conditions. Medication should never replace evaluation and counseling on modifiable factors.[5]

Because current research on galactogogues is relatively inconclusive and all of the agents have potential side effects, the Academy of Breastfeeding Medicine (ABM) does not recommend any specific pharmacological or herbal galactogogues.[5] Clinicians should prescribe galactogogues with appropriate caution with regard to drug-to-herb interactions as well as to an overall risk-to-benefit approach. Close follow-up of both mother and baby is essential to monitor the status of lactation as well as any adverse events of the drug(s) on mother or infant.[5]

The herbs were usually recommended as a last resort when other non-pharmacological measures have not resulted in an increase in milk volumes.[5] A number of botanical remedies (*e.g.* anise, fennel fenugreek seed, nettle and milk thistle) have been traditionally used to stimulate milk production.[4] The available studies on herbs, herbal medicines, or herbal galactogogues suffer from the same deficiencies as the studies for pharmacological agents: small numbers of subjects, poorly defined eligibility criteria, lack of information regarding breastfeeding advice, lack of randomization, control and blinding (levels of evidence II-1,31 II-332).[5,14]

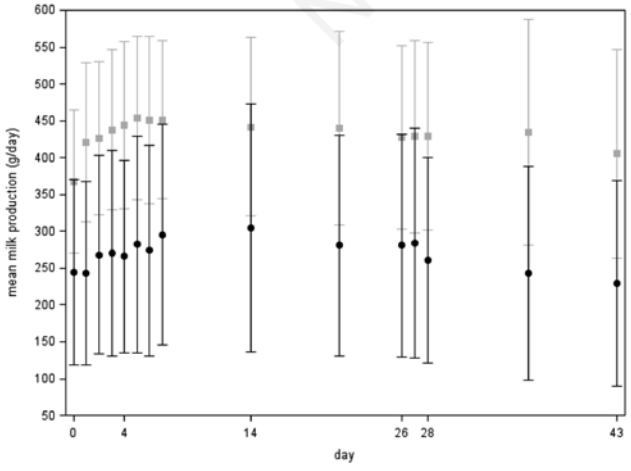

Figure 3. Average milk production (g/day) and 95% confidence intervals in silymarin-guess (grey squares) and placebo-guess (black dots) groups.

Four reviews were recently published on the topic of galactogogues. All the reviews analyzed the safety and efficacy of herbal remedies used as galactogogues during lactation. All the studies analyzed in these reviews enrolled only term mothers at different lactating stages. All of them concluded that no current recommendation is made for the use of herbs as galactogogues, and well-designed and well-conducted clinical trials are needed to generate evidence on this topic.[15-18]

Recently, the observation that an extract of milk thistle increases lactation in cows,[11] rats[12] and women[13] has gained renewed interest in its galacto-genic properties.

In this double blind clinical trial we studied silymarin, an active ingredient known for many years as a supposed protective liver agent, due to its possible galactogogue properties. The product was tested and administered in the form of BIO-C® (420 mg/day). Currently our study is the first trial focused on mothers who delivered preterm. The present study was preceded by an investigation aimed at evaluating the tolerability, as well as the total absence of silymarin in milk of women taking a dose of 420 mg/day in the form of BIO-C®.

Our study does not show a difference, neither in primary nor in secondary endpoints between the two arms (silymarin *vs* placebo).

In Di Pierro's study, women orally treated for 63 days with silymarin showed a clear galactogogue role for the product with an increase of about 86% of the daily milk production (placebo: +32%), but the eligibility criteria are not clear: gestational age at delivery and age of neonates are not indicated. In this study the healthy women enrolled were considered borderline in terms of normal daily milk production.

In our study we included only preterm mothers who had delivered at gestational age <32 weeks; the assumption was that these women would present hypogalactia due to breastfeeding difficulties related to an incomplete lactation process and to higher stress levels. However, the greatest difficulty that preterm mothers found during breastfeeding was not only to initiate lactation, but also to maintain an adequate volume of milk over time. Additionally, a difference in the amount of milk production at baseline was present (although not statistically significant) between the two arms, *i.e.* women in the silymarin arm were found to express a higher amount of milk with respect to women in placebo arm. This finding may be explained by the fact that the silymarin arm included a higher percentage of multiparae subjects, with a history of prolonged breastfeeding duration in previous pregnancies: actually, preterm mothers with a previous experience of breastfeeding are reported to produce a higher amount of milk over time.[19]

No side effect was reported in both groups. Furthermore, silymarin was not tracked in collected milk samples, which supports the safety of silymarin both for mothers and neonates. Future studies should therefore be performed with a specific focus on mothers affected by real hypogalactia. However, it is quite difficult to define a cut-off to diagnose hypogalactia in quantitative terms, because this condition represents a variable and dualistic concept that can be better defined as a quali-quantitative inadequacy to meet the child's needs over time.

Conclusions

Breastfeeding importance in the first months of life is undeniable, especially in preterm neonates. However, mothers often need to increase their milk production and when other non-pharmacological measures fail, galactogogues can be recommended. Off-label galacto-

gogue agents, such as domperidone and metoclopramide, however, can have serious side effects, which compromise their use.

In this double-blind randomized clinical trial, we investigated the use of silymarin as a galactogogue for mothers having delivered preterm infants. No significant difference was found in milk production in the period of 26th-28th day of assumption. No side effects were reported in both groups. Furthermore, silymarin was not found in milk samples collected.

Further investigation on specific patient groups affected by hypogalactia, defined according to strict criteria, has to be planned to assess the efficacy of the product in increasing milk production.

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