ORIGINAL ARTICLE

Clinical efficacy, safety and tolerability of BIO-C® (micronized Silymarin) as a galactagogue

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Abstract. *Background and aim of the work*: The authors have previously reported the use of Silymarin (a *Silybum marianum* standardized extract) as a promoter of milk production in cows. Due to the important psychological impact of hypogalactia in women after delivery, we evaluated the role of Silymarin as a safe and effective galactogogue for human species. *Methods:* 50 healthy women during lactation were enrolled in order to verify the galactogogue role played by an oral treatment with micronized Silymarin (420 mg/day) in comparison with an undistinguishable placebo product. *Results*: Women orally treated for 63 days with Silymarin showed a clear galactagogue role for the product with an increase of 85.94% of the daily milk production (placebo: +32.09%). No drop out, nor unwanted effects were reported in both groups. Compliance and tolerability were also very good. *Conclusions*: Silymarin may be considered as a safe and effective herbal product that can be orally administered in order to improve the daily milk production in healthy women after delivery, without affecting milk quality. (www.actabiomedica.it)

Key words: Silymarin, galactagogue, BIO-C®

Introduction

The study of biologically active substances from plants is continuously improving and frequently puts into evidence the potential use of these fractions in the medical praxis.

A pharmacological action verified in the veterinary praxis can sometimes bring to the same evidence in the human one. Such examples are for instance the anti-coagulant effect of the *Melilotus officinalis*, due to the content of coumarin polymeric derivatives (1), and the dewormer action of Lespedeza herb (2), firstly observed in cow and goat respectively.

Similarly, recent studies have shown a potential galactagogue effect of silymarin in milk production in cows (3). These studies demonstrated an average increase of 5-6 L/die per cow during lactation after the treatment with 10 g/die/cow of Silymarin extract. In spite of the increased milk quantity, cows were more healthy than the untreated animals and their Body Mass Index (BMI) improved reducing the need for medical care, a very common need felt from the farmers for many weeks after delivery. Since then, some authors have published on the use of Silymarin extract in cows during lactation demonstrating a significant reduction of acetone and acetoacetic acid both in blood and in milk, anticipating data concerning product safety in animals in spite of its high and chronic use. In particular these studies showed the properties of Silymarin in significantly reducing blood β-hydroxybutyrate acid (BHBA) as well as ketonuria, showing a positive effect on their organic and body conditions (4).

Silymarin is an extract, characterized by the presence of a highly standardized flavanolignans fraction, obtained from *Silybum marianum*. 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 (5). The use of such

plant is known since the ancient times and it has been reported in the Holy Bible, and also evidenced by Teofrasto (IV century b.C.), Plinio (I century a.C.), Dioscoride (I century a.C.) and Mattioli in 1500 a. C. The extract obtained from *Silybum marianum* has been often used in the clinical praxis for treating several dysfunctions such as acute and chronic hepatitis (cirrhosis included) of different aetiology, including toxins, drugs, viruses and alcohol. The product, in form of medicinal speciality and/or nutritional supplement, is nowadays commonly used all over the world for the same purpose. The most active ingredient of Silymarin is, in all likelihood, a mixture of flavanolignans precisely known as silycristine, silybin, isosilybin and silydianin in their several diastereoisomeric forms. These are products generated by the oxidative combination of a lignan and a flavanoid and corresponds to 60-80% of the extract. The most abundant and active component of this mixture is surely silybin, available in antivenin centres as an injectable LPS-free product for the treatment of intoxication from *Amanita phalloides*.

The possible Silymarin mechanisms of action (5-8), explaining its liver protective action are listed as follows: inhibition of the bound between toxins and hepatocytes; reduction of the glutathione oxidation with increase of its hepatic level; stimulation of the ribosomal RNA-polymerase with increase of the liver protein synthesis; inhibition of the lipidic peroxidation in hepatocytes of ribosomal membranes; inhibition of DNA damage due to peroxide and superoxide anion; inhibition of phosphodoesterase; inhibition of type III procollagen formation, with interference with the liver fibrosis process; inhibition of the synthesis of chemical mediators involved in inflammation (α-TNF, LTB4, PGE2); P-glycoprotein modulator.

The regenerative effect of Silymarin on hepatocytes seems to be selective towards healthy cells, since Silymarin does not promote neither the proliferation of normal liver cells in vitro nor the proliferation of other malignant cell lines. Pharmacokinetic data on Silymarin report that the compound is poorly absorbed after oral administration with only a 23-47% of bioavailability. Plasma peak concentration occurs after 2-4 hours of administration. Half-life lasts about six hours. Silymarin, after absorption from gut mucosa, passes through by the portal circulation reaching the liver where it exerts most of its pharmacological action. Because of this, the concentration in the bile is often much higher than in the serum. In terms of excretion only 2-5% is excreted through urine, being 20-40% excreted by the bile in the form of glucuronide and sulphate. Silymarin extract shows an excellent safety profile at therapeutic doses and, both alone and associated with standard traditional therapy, it improves the clinical course and survival rate in case of acute or chronic hepatitis induced by drugs, toxins, viruses and alcohol (9).

The aim of the our study was to demonstrate that the galactagogue action, already observed and reported in cows with Silymarin, was also present in the human species.

After checking the total absence of the product in the mother's milk, a detailed protocol was drawn in order to demonstrate and verify the galactagogue effect of micronized Silymarin in healthy lactating women. The use of plant and drug products in enhancing lactation is more likely widespread than assumable (not always in perfect agreement with the referent medical doctor), and numerous papers have been published in the medical literature claiming the efficacy of various products. Many herbals and drugs, such as oxitocin and dopamine antagonists, have been reviewed but a few efficacy proofs and incomplete data concerning toxicology have been found (10).

Materials and methods

In order to verify the total absence of milk flavanolignans after oral administration of Silymarin in lactating woman we enrolled at the San Marcos Hospital in Lima (Perù), and in agreement with the Ethical Council of the same hospital, 5 healthy lactating volunteers who had decided to stop lactation due to the age of newborns (9 months old). They were orally administered 3 times a day with an oral dose of 600 mg of micronized Silymarin. After 5 days their milk was collected and analyzed by HPLC (11). For the analytical control a Silymarin reference standard was kindly provided by Indena (Milano, Italy). According to their certificate of analysis this reference standard

was constitueted by: Silybin A (13.1%), Silybin B (21.6%), Isosilybin A (6.6 %), Isosilybin B (3.9%), Silydianin (9.4%), Silychristin (15.5%), Isosilychristin (1.5%), Taxifolin 2.2%).

In order to verify the galactagogue effect of micronized Silymarin, we enrolled, at the San Marcos Hospital in Lima (Perù) and in agreement with the Ethical Council of the same hospital, other 50 volunteers during lactation who, after psychological, medical, pharmacological and objective examination, did not present anomalies or diseases to invalidate the trial. In spite of the evident healthy conditions, the enrolled women were considered borderline in terms of normal daily milk production, at least in comparison with the historical recordings of the same hospital claiming, as compared with the same type of lactating woman (identical in terms of age, weight, newborn's age and number of children), a daily milk volume of around 700 mL/day. The values of the 50 enrolled women was not anyway statistically different from this recording. As a matter of fact the average value at t=0 for the tested group was 601,92 mL/day and the one for the placebo group was 530,36 (Table 1).

In order to keep the two groups as healthy and homogeneous as possible, during the study all mothers received the same diet that was typical for healthy women during lactation (about 2600 Kcal/die).

Participants were divided into two groups of 25 subjects considering their age, number of sons and age of the last born. The two groups received either BIO-C® (micronized Silymarin, 420 mg/day) or placebo (undistinguishable from the active), respectively. The dose was decided on the basis of the current pharmaceutical dosage established in most Countries of the world where Silymarin, being a pharmaceutical product, is orally given at 420 mg/die. The treatment lasted 63 days. In order to verify the group homogeneity at t=0, we used the Student's *t* test. No significance in the mean values of the three considered parameters was observed: age of the mother, age of the last born and number of sons for each mother. In order to verify statistically significant differences in terms of milk quantity produced, we used Student's *t* test; on the contrary, in order to verify statistically significant changes in terms of milk quality (water, sugars, fats, proteins), we used the Mann-Whitney *U* test. The study began on day 0, when women assumed BIO-C® or placebo for the first time. During the following 24 hours each mother was monitored by qualified personnel with regards to the established quantitative records and samples necessary for analysis. The used method considered both the milk sucked by the newborn (double weighing, before and after sucking) and the milk taken with the breast-pump after each sucking to void the gland. Moreover, a portion of the milk sample was used to carry out the quantitative analysis of water, fats, proteins and carbohydrates. In this way we obtained the qualitative and the quantitative profile on day 0 of the 50 mothers before administering the active (25 mothers) and the placebo (25 mothers) (Table 1). During the following 63 days all mothers of the two groups were orally administered BIO-C® or placebo.

BIO-C® is currently commercially packaged in 5.5 g single dose/sachet (product brand: PiùLatte®). For the clinical trial a "brand less" product was used. Placebo, refined sugars with no difference in terms of weight, colour and taste from the active drug, was proposed with the same packaging.

On days 30 (Table 2) and 63 (Table 3) each mother was monitored with regards to established quantitative records and samples necessary for biochemical analysis.

Food-grade Silymarin, extracted by food-grade solvents, was kindly provided by Indena S.p.A. (Milano, Italy); micronization and packaging technology were applied by the manufacturer of the finished product (S.I.I.T. s.r.l.; Trezzano S/N, Milano, Italy). The tested product and the placebo were kindly provided by Milte S.p.A. (Milano, Italy).

Results

HPLC analysis has shown that Silymarin flavanolignans were undetectable in milk after 5 days of administration of 600 mg of Silymarin 3 times a day. The method, taken from the European Pharmacopoeia (11), detects Silymarin flavanolignans with a limit of about 1 ng/ml. According to this detection limit, no milk peak of Silymarin component has been detected in our tests.

According to the HPLC method used, the minutes elapsed to detect components are: Taxifolin about 10 min, Isosilychristin about 12 min, Silychristin about 13 min, Silydianin about 15 min, Silybin A about 27 min, Silybin B about 30 min, Isosilybin A about 34 min, Isosilybin B about 35 min. The HPLC analysis of the milk collected from the 5 healthy volunteers have clearly shown no peaks at all, but the reference standard, until '60 min.

In terms of galactagogue effect, as reported in Table 1, the mean quantity of produced milk at day=0 was 601.92 g for BIO-C®, and 530.36 g for the placebo. For both groups the standard deviation was inferior to 15%. As also reported in Table 1, no difference was observed concerning the chemical milk composition between the 2 groups. This means that before beginning BIO-C® or placebo the 50 mothers produced similar milk from a quantitative and qualitative point of view.

At day 30 (Table 2), the results clearly demonstrated a stronger increase in the average quantity of milk produced by mothers treated with BIO-C® (989.76 g) than in the quantity of milk produced by mothers treated with Placebo (649.76 g). Even in this case for both groups the standard deviation was inferior to 15%.

Table 1. Qualitative and quantitative milk profile on day 0[(1)]

	BIO-C® Group Mean±SD	Placebo Group Mean±SD
Milk (g)	601.92±65.12	530.36±69.37
Water (%)	86.8408±0.20	86.7816±035
Fats (%)	3.6632±0.3222	3.3540±0.2985
Carbohydrates (%)	7.0816±0.0911	7.1536±0.1095
Proteins (%)	1.1884±0.0217	1.1652±0.01899

(1) The difference is not significant

Table 2. Qualitative and quantitative milk profile on day 30[(1)]

	BIO-C® Group Mean±SD	Placebo Group Mean±SD
Milk (g)	989.76±102.33	649.76±78.35
Water (%)	87.8784±0.6345	87.1820±0.7562
Fats (%)	2.5620±0.1453	2.6604±0.1759
Carbohydrates (%)	7.3920±0.0041	7.3960±0.037
Proteins (%)	1.1328±0.0923	1.2040±0.0976

(1) The difference between values is significant for milk (g) ($p< 0.01$)

Table 3. Qualitative and quantitative milk profile on day 63[(1)]

	BIO-C® Group Mean±SD	Placebo Group Mean±SD
Milk (g)	1119.24±115.89	700.56±95.66
Water (%)	87.4888±0.572	86.9156±0.498
Fats (%)	2.9192±0.1342	3.0648±0.1981
Carbohydrates (%)	7.8128±0.3241	7.5648±0.3562
Proteins (%)	1.1260±0.0983	1.2268±0.0923

(1) The difference between values is significant for milk (g) ($p<0.01$)

It is also interesting to consider not only the quantity of produced milk in terms of absolute value but also in percentage the increase for each mother compared to day 0, which was 64.43% in the treated group and 22.51% in the placebo group.

As concern biochemical analysis, the results showed no difference between the 2 groups and all chemical parameters were within the normal range: the administration of BIO-C® did not alter the physiological values of the mother's milk composition.

At day 63 (Table 3), results confirmed what was already seen on day 30: the mean quantity of milk produced by mothers treated with BIO-C® (1119.24 g) was significantly higher than the quantity produced by mother treated with placebo (700.56 g). Even in this third case, for both groups the standard deviation was inferior to 15%.

Also in this case it is interesting to consider not only the quantity of produced milk in terms of absolute value but also the percentage increase for each mother compared to day 0 (85.95% and 32.09% respectively). The analysis of biochemical parameters (water, fats, carbohydrates and proteins) recorded on day 63 confirmed that values do not change from the physiological standards of mother's milk. During the study no single drop out was recorded in both groups and compliance and tolerability observed by clinicians and reported by the mothers of both groups were very good.

Conclusions

In this clinical trial we studied Silymarin, an active ingredient known for many years as a protective liver agent, due to its possible galactagogue properties.

The product was tested and administered in the form of BIO-C® (420 mg/die). Before packaging, the active drug was micronized, a procedure that improves its poor oral bioavailability.

The present study was preceded by an investigation aimed at evaluating the tolerability and the total absence of Silymarin in women's milk taking a dose of 1800 mg/die in the form of BIO-C®.

As shown in the results section, the recorded data in this trial proved that BIO-C® shows an effective and determinant galactagogue propriety.

The action played by the micronized product, administered for 63 days, induced, as observed at days 30 and 63, a high increase of the quantity of milk production. The observed increase occurs without affecting the main milk biochemical characteristics (proteins, sugars, lipids, water).

At the end of the study we recorded in the BIO-C® group an increase, from day 0 to day 63, of 85.94% compared to a mean increase of 32.09% in the placebo group. Our idea is that the increase observed in the placebo group could be explained by the role played by the breast pump which was already seen in our experience, but other possible effects (spontaneous trend, psychological, adaptative, reflex effects) cannot be excluded.

It is very difficult to assume a possible mechanism of action for the product. After delivering a calf, a cow is subject to a decrease in terms of organic and body conditions, in which a reduced ability in producing milk is just one of the parameters to be considered: weight loss, BMI reduction, liver pathology, mastitis, abomasal displacement, medical care, just to mention a few. This worsening in terms of body conditions may explain, at least in part, why Silymarin, administered after calf delivery, works. As a matter of fact, treatment with Silymarin helps the cow body functions, liver included. The animal eats frequently, does not lose weight, has a better liver functionality and a lower pathology incidence. As a result, the animals produce more daily milk in comparison with untreated cows. Since humans undergo a total different situation, an alternative explanation has to be found. Starting from some experimental results showing the role of weak estrogens played by flavanolignans in tumour mice models (12), it can be assumed that Silymarin, by reducing the estrogenic value, promotes, or at least increases, lactation. Some other, still unpublished, results show that administration of the product in rats determines a clear increase in prolactin production and secretion. The same investigation in humans could give a possible definitive answer in terms of how and why Silymarin oral administration in healthy lactating women increases daily milk quantity without affecting its chemical composition.

In conclusion, further investigation on larger patient groups have also to be planned to confirm the efficacy of the product in increasing milk production and to confirm the galactagogue properties of the product in women with both mild/partial hypogalactia, like the ones tested in the study, and in women with the most severe form, like those which deliver preterm newborns.

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