Received: 22 December 2016 | Accepted: 29 May 2017

DOI: 10.1111/nmo.13145

ORIGINAL ARTICLE

WILEY Neurogastroenterology & Motility NGM

Efficacy of a standardized extract of *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *Lactobacillus acidophilus* (HA122) in infantile colic: An open randomized controlled trial

M. Martinelli[1] | D. Ummarino[1] | F. P. Giugliano[1] | E. Sciorio[1] | C. Tortora[1] | D. Bruzzese[2] | D. De Giovanni[2] | I. Rutigliano[2] | S. Valenti[3,4] | C. Romano[3,4] | A. Campanozzi[2] | E. Miele[1] | A. Staiano[1]

[1]Department of Translational Medical Science, Section of Pediatrics, University of Naples "Federico II", Naples, Italy

[2]Department of Public Health, University of Naples "Federico II", Naples, Italy

[3]Institute of Pediatrics of the University of Foggia, Foggia, Italy

[4]Endoscopy and Gastroenterology Unit, Department of Pediatrics, University of Messina, Messina, Italy

Correspondence
Annamaria Staiano, MD, Department of Translational Medical Science, Section of Pediatrics, University of Naples "Federico II", Naples, Italy.
Email: staiano@unina.it

Funding information
No external funding for this manuscript.

Abstract

Background: Infant colic (IC) is a prevalent physiological event of infants, which can disrupt the child's home environment. We aimed to investigate the effectiveness of a mixture of *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *Lactobacillus acidophilus* (HA122) compared with *Lactobacillus reuteri* DSM 17938 and with simethicone for the treatment of IC.

Methods: A multicenter randomized comparative study was conducted in infants with colic, according to Rome III criteria, who were randomly assigned to receive *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) (Colimil® Plus®; Milte Italia Spa, Milan, Italy) (Group A), *L. reuteri* DSM 17938 (Group B) and simethicone (Group C). Treatment was given to subjects for 28 days.

Key Results: One-hundred and seventy-six patients completed the study. Mean daily crying time at day 28 was significantly lower in group A (−44, 95% CI: −58 to −30, *P*<.001) and group B (−35, 95% CI: −49 to −20, *P*<.001) when compared to group C. No significant difference was observed between Group A and Group B (mean difference: −9 minutes, 95% CI −23 to +5, *P*=.205). At day 28, 39 of 57 (68.4%) of infants in Group C responded to the treatment compared with 57 out of 60 patients (95%) of Group A and 51 out of 59 (86.4%) of Group B (*P*<.001).

Conclusions: This study suggests that administration of *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) and *L. reuteri* DSM 17938 are significantly more effective than simethicone in IC. Clinical Trial Registration: ClinicalTrials.gov: NCT02708238.

KEYWORDS

complementary and alternative medicine, herbal supplement, infantile colic, probiotics

Abbreviations: CAM, complementary and alternative medicine; GI, gastrointestinal; IC, infantile colic; RCTs, randomized controlled trials.

1 | INTRODUCTION

Infantile colic (IC) is a common condition occurring during the first 4 months of life and reaching up to 20% of infants.[1,2] It is usually defined on the basis of Rome III criteria and the diagnosis must include all of the following in infants from birth to 4 months of age: paroxysms of irritability, fussing, or crying that start and stop without obvious cause; episodes lasting 3 or more hours per day and occurring at least 3 days/week for at least 1 week; no failure to thrive.[3] Despite the high prevalence, the pathogenesis of IC remains elusive. Gastrointestinal (GI), psychosocial, and neurodevelopmental disorders have been suggested.[4] Although benign and self-limiting, IC is associated with maternal depression,[5] early breastfeeding cessation,[6] and shaken baby syndrome.[7] As a matter of fact, the total annual cost of infant crying and sleeping problems in the first 12 weeks has been estimated to £65 millions in United Kingdom (US$104 millions).[8] Although the mainstay of IC management is still limited to the support and the reassurance of the parents,[9] these findings highlight the need for new therapeutic strategies. The use of probiotics is now emerging as a promising strategy for the treatment of IC.[10,11] However, few strains have been tested and results from randomized controlled trials (RCTs) and systematic reviews are conflicting.[12–14] Although no evidence of benefits has been reported in clinical trials, the use of simethicone is widespread in clinical practice, more frequently through self-medication.[14] The paucity of treatment options and dissatisfaction with conventional health care may lead parents to seek out complementary and alternative medicine (CAM) options for their infants.[15] Particularly, the efficacy of a herbal formula containing *Matricaria recutita* L., *Foeniculum vulgare* M. var. dulce and *Melissa officinalis* has been previously demonstrated in IC.[16] Recently, the manufacturer of the product, in order to potentially increase its efficacy, decided to add to the herbal formula the beneficial properties of a tyndallized probiotic (*Lactobacillus acidophilus* HA122; Colimil® Plus®; Milte Italia Spa, Milan, Italy). The process of tyndallization consists in a heat treatment for 1 hour at 70°C on three consecutive days and by gamma irradiation of lactobacilli.[17] This procedure guarantees the killing of the live bacteria and the preservation of probiotic-produced, soluble factors, so called postbiotics, which can elicit the beneficial effects.[18] A postbiotic could therefore be a safer alternative to the use of whole bacteria avoiding the risks associated with the administration of live bacteria.

Nevertheless, the evidence supporting the use of CAM for IC management is still very limited to date.[15,16,19] The primary aim of this study was to investigate the effectiveness of a standardized extract of *Matricariae chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) compared with Lactobacillus reuteri (DSM 17938; Reuterin®; NOOS Srl, Rome, Italy) and with simethicone (Mylicon®; Johnson & Johnson Spa., Pomezia, Italy) for the treatment of IC; secondary aims were to evaluate the safety and tolerability of the treatments.

Key Points

- Infantile colic is a common condition occurring during the first 4 months of life and reaching up to 20% of infants.
- The administration of *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *L. acidophilus* (HA122) and *L. reuteri* DSM 17938 was significantly more effective than simethicone in improving colic symptoms.
- The use of a mixture of herbal supplements and tyndallized probiotics may represent a new therapeutic strategy in the management of colicky infants.

2 | MATERIALS AND METHODS

This was a prospective, multicentre, open-label, randomized, controlled trial. All consecutive infants diagnosed with IC, according to Rome III criteria, were prospectively enrolled between April 2014 and July 2015 by three different Pediatric Gastroenterology units: Department of Translational Medical Science, Section of Pediatrics, University of Naples "Federico II"; Institute of Pediatrics of the University of Foggia; Endoscopy and Gastroenterology Unit, Department of Pediatrics, University of Messina. Study participants met the following inclusion criteria: diagnosis of IC according to Rome III criteria[3]; age ≥2 weeks to 4 months; breastfed or fomula-fed infants; term delivery (≥37 weeks gestation at birth); 5-minute Apgar score ≥7; and birth weight ≥2500 g. Exclusion criteria included a major medical problem or acute illness; a family history of atopy; and history of antibiotic treatment or probiotic supplementation before or during the study. After the enrollment, all children were randomly assigned to receive: a standardized extract of *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) administered at the dose of 1 mL twice a day of a commercially available solution (2 mL of solution contains 18 mg of *M. chamomilla* L., 130 mg of *M. officinalis* L. and 2×10^9 tyndallized *L. acidophilus* cells [HA122]) (Group A); *L. reuteri* DSM 17938 administered at the dose of 10^8 colony-forming units/day in 5 drops of a commercially available oil suspension (Group B); simethicone given at a dose of 60 mg in 15 drops 2 times per day of a commercially available solution (Group C). The manufacturers did not supply the products for the study. Treatment was given to subjects for 28 days. The study's primary outcome was the mean daily crying at the end of treatment (day 28). The secondary outcome measure was the number of participants who responded to treatment on day 28. Infants who experienced a decrease in the daily average crying time of 50% from baseline were considered as responders to the treatment. Parents were instructed to complete a structured 28-day maternal diary, modified from Barr et al. in order to record the frequency of colic episodes and the daily crying time (in minutes), feeding schedule, stool frequency and characteristics, and any adverse events experienced.[20] Follow-up visits were performed at each involved unit and conducted on study days 7, 14, 21 and 28

by the same study investigator. In addition at each visit diaries unused study products were returned to measure the adherence to the study.

2.1 | Ethical considerations

The Institutional Review Board of the University of Naples "Federico II" approved the study protocol with the registration number 234/13. Written, informed consent was obtained from parents of all the enrolled children.

2.2 | Statistical analysis

Demographic and clinical data referred to the baseline visit were summarized using standard descriptive statistics and compared between group (without reporting statistical significance) to assess whether good balance of baseline characteristics was achieved by randomization. Longitudinal trajectories of daily crying time during the follow-up period, were analyzed by using random-intercept linear mixed model (LMM) in which time from baseline was treated as categorical factor (four levels: 7, 14, 21, and 28 days) to account for non-linear relationships. Baseline measurement of daily crying duration was added to the model as adjustment covariate. Results of LMMs were reported as estimated marginal means with the corresponding 95% confidence intervals (95% CI). Differences among the three treatment arms were estimated by adding in the LMM an interaction term between group and time; the corresponding *P*-values were adjusted using Tukey Method. Mean daily crying at day 28 was also compared between infants exclusively or partially breastfeeding and children exclusively formula-fed separately in each treatment arm by using general linear model adjusted for baseline measurement. Response rates were compared between groups using chi-squared test and further analyzed by computing relative risk (RR) with the corresponding 95% CI. In order to adjust for baseline measurement, a Poisson Regression with robust variance estimation was used.[21] Statistical significance was predetermined as $P<.05$. SPSS version 15 (SPSS Inc, Chicago, Illinois, USA) and R statistical platform (version 3.1, R Foundation for Statistical Computing, Wien, Austria, https://www.R-project.org/) were used for all statistical analyses. A sample size of 50 children in each group allowed to detect a difference of at least 50 minutes in daily average crying time among the three groups with a power of 0.8 and a two-sided alpha of 0.017 adjusted for multiplicity. The anticipated standard deviation was equal to 75 minutes. Assuming a drop-out rate of 20%,[22] 60 infants per group were needed. In order to guarantee the allocation concealment, an independent statistician, unaware of the enrolled patients, prepared and kept a computer-generated three-treatment randomization schedule with random block of varying size to maintain balance in the allocation of participants between treatment arms. Randomization was stratified by type of infant feeding (exclusively breastfed or formula-fed) and age. At each enrollment, the statistician, who was the only one to have access to the randomization schedule, communicated to the main investigators the drug to be prescribed. All analyses were conducted both on intention to treat (ITT) analysis including all patients randomized in the groups and on a per protocol basis including all patients in the groups who completed the expected treatment. In the ITT analysis, missing data were imputed using multiple imputation method and nine different datasets were generated. As the two analysis gave high consistent results, only the per protocol analysis will be reported.

TABLE 1 Baseline clinical and demographic characteristics of the enrolled infants

Variables	Group A (n=60)	Group B (n=60)	Group C (n=60)
Age, days (mean±SD)	39.3±20	37.4±14.7	34.1±13.3
Male Gender (n, %)	29 (48.3)	38 (63.3)	19 (31.7)
Vaginal delivery (n, %)	39 (65)	38 (63.3)	38 (63.3)
Birth weight, g (mean±SD)	3239±291.3	3279.9±383.3	3081.7±576.3
Gestation age, weeks (mean±SD)	38.7±1	38.7±0.9	38.7±0.9
Weight, g (mean±SD)	4232.8±837.4	4562.8±706.5	4488.2±698.4
Feeding (n, %)			
Exclusively breastfeeding	45 (75)	43 (71.7)	45 (75)
Partial breastfeeding	5 (8.3)	6 (10)	5 (8.3)
Exclusively formula-fed	10 (16.7)	11 (18.3)	10 (16.7)
Mean daily crying±SD, min	235±25.4	235.1±35.4	230.1±28.6

Group A: Infants treated with *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *L. acidophilus* (*HA122*).
Group B: Infants treated with *L. reuteri DSM 17938*.
Group C: Infants treated with simethicone.

3 | RESULTS

Two-hundred patients diagnosed with IC, according to Rome III criteria, were prospectively screened between April 2014 and July 2015. Twenty patients were subsequently excluded from the study for the following reasons: declined to participate (n=8); acute illness (n=5); antibiotic therapy (n=1); and probiotic supplementation (n=6). One-hundred and eighty patients were included in the study and all children were randomly assigned to receive *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) (Group A; n=60), *L. reuteri* DSM 17938 (Group B; n=60) or simethicone (Group C; n=60). Baseline clinical and demographic characteristics of the enrolled patients are showed in Table 1. One patient of Group B and three patients of Group C were lost at follow-up. A flow diagram showing the subjects' progression through the study is reported in Figure 1. One-hundred and seventy-six patients completed the whole follow-up and were finally included in the analysis. Linear mixed model, adjusted for baseline crying duration, showed that mean daily crying time at day 28 was significantly lower in group A (−44, 95% CI: −58 to −30, *P*<.001) and group B (−35, 95% CI: −49 to −20, *P*<.001) when compared to group C (Table 2; Figure 2). No significant difference was observed between Group A and Group B (mean difference: −9 minutes, 95% CI −23 to +5, *P*=.205; Table 2; Figure 2). By examining the whole follow-up period, it emerged that differences in mean daily crying time between Groups A and C become statistically significant from day 7, while statistical significance in the comparison between Group B and Group C was reached from day 14 onward (Table 2). At day 28, 39 out of 57 (68%) of infants in Group C responded to the treatment compared with 57 out of 60 patients (95%) of Group A and 51 out of 59 (86%) of subjects of Group B (*P*<.001 by chi-squared test). After adjusting for baseline crying duration, the RR for a treatment success was equal to 1.39 (95% CI: 1.15-1.67, *P*=.001) in group A with respect to group C and 1.26 (95% CI: 1.03-1.54, *P*=.026) in group B with respect to Group C. No difference in rate of responders was observed between Group B and Group A (RR=0.91, 95% CI 0.81-1.02, *P*=.114). Figure 3 shows percentages of responders at different time points according to the treatment arm. Considering infants' feeding, no significant differences were observed in primary or secondary outcomes between infants exclusively or partially breastfeeding and children exclusively formula-fed in all the three groups of treatment. In particular, after

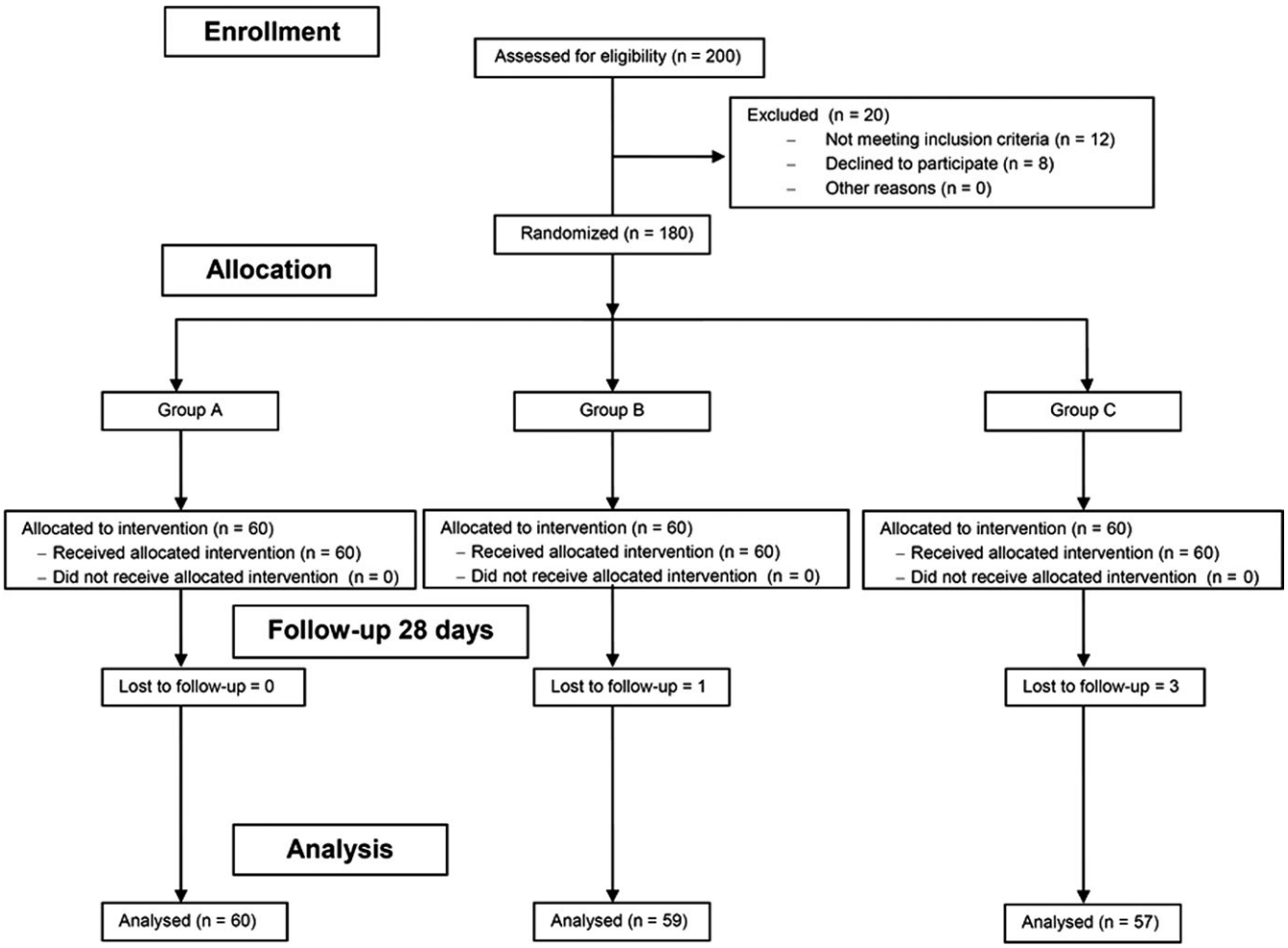

FIGURE 1 Flow diagram of the subjects' progression through the study. Group A: Infants treated with *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *L. acidophilus* (HA122); Group B: Infants treated with *L. reuteri* DSM 17938; Group C: Infants treated with simethicone

TABLE 2 Primary outcome of the study

	Mean duration of crying (min/day)		
Time	**Group A**	**Group B**	**Group C**
Day 7	166 [156-176]	177 [167-187]	188 [177-198]
Day 14	123 [113-133]	140 [130-150]	160 [150-170]
Day 21	78 [68-88]	92 [83-102]	127 [117-137]
Day 28	53 [43-63]	62 [52-72]	97 [87-107]

	Group A vs Group C		Group A vs Group B		Group B vs Group C	
Time	**Mean diff. [95% CI]**	***P***	**Mean diff. [95% CI]**	***P***	**Mean diff. [95% CI]**	***P***
Day 7	−21 [−36 to −7]	.009	−11 [−25 to 3]	.302	−11 [−25 to 3]	.297
Day 14	−37 [−51 to −23]	<.001	−17 [−31 to −3]	.053	−20 [−35 to −6]	.016
Day 21	−49 [−63 to −35]	<.001	−14 [−29 to 1]	.115	−35 [−49 to −20]	<.001
Day 28	−44 [−58 to −30]	<.001	−9 [−23 to 5]	.413	−35 [−49 to −20]	<.001

Group A: Infants treated with *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *L. acidophilus (HA122);* Group B: Infants treated with *L. reuteri DSM 17938*; Group C: Infants treated with simethicone.
All results are based on a random-intercept linear mixed model adjusted for baseline crying duration. *P*-values were adjusted using Tukey's method.
CI: confidence intervals; Diff: difference.

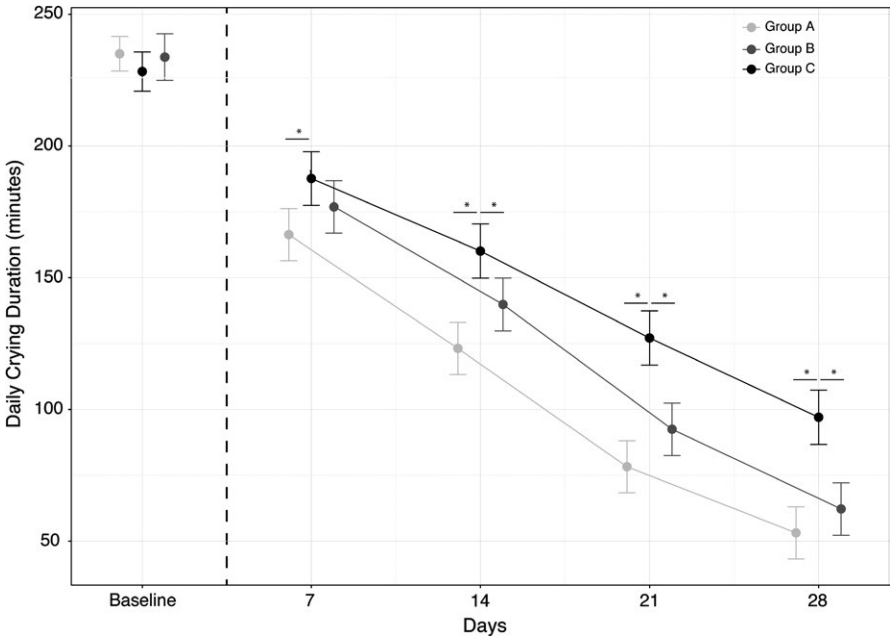

FIGURE 2 Longitudinal trajectories of daily crying time among the three different treatment's groups during the follow-up period, analyzed using random-intercept linear mixed model (LMM). Results of LMMs are reported as estimated marginal means with the corresponding 95% confidence intervals. Group A: Infants treated with *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *L. acidophilus* (HA122); Group B: Infants treated with *L. reuteri* DSM 17938; Group C: Infants treated with simethicone

adjusting for baseline crying duration, mean difference in daily crying time between infants exclusively or partially breastfeeding and children exclusively formula-fed was equal to 5 minutes (95% CI −22 to +31, *P*=.721) in Group A, −12 minutes (95% CI −37 to +16, *P*=.428) in group B, and +18 minutes (95% CI −9 to +46, *P*=.189) in Group C. Moreover, the RR of experiencing a decrease of 50% from baseline in the daily crying time between infants exclusively or partially breastfeeding and children exclusively formula-fed was equal to 0.94 (95% CI 0.88-1.01; *P*=.084) in group A, 1.1 (95% CI 0.79-1.53; *P*=.57) in group B, and 0.853 (95% CI 0.57-1.27; *P*=.433) in Group C. No significant difference in stool patterns was observed among the three study groups.

3.1 | Adverse events and adherence to the treatments

No adverse event was reported in any group. The adherence to the treatment was not significantly different among the three arms (Group A: 58/60 [96%]; Group B: 55/59 [93.2%]; Group C: 52/57 [91%]; *P*=.1).

4 | DISCUSSION

To the best of our knowledge, this is the first RCT, which demonstrates the efficacy of a standardized extract of *M. chamomilla* L., *M. officinalis*

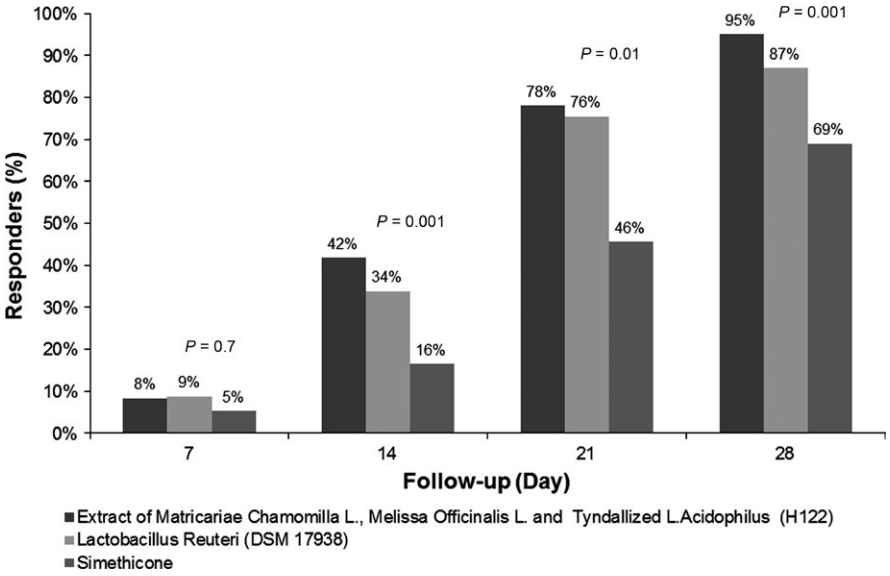

FIGURE 3 Percentage of responders in each group on days 7, 14, 21, and 28 on the per protocol analysis. Infants were classified as responders if they experienced a decrease in the daily average crying time (in minutes) of 50% from the baseline measurement

L. and tyndallized *L. acidophilus* (HA122) for the treatment of IC. The results of the present study indicates that the administration of *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) twice a day significantly reduced infant crying time at 28 days when compared with simethicone in infants, showing the same efficacy of *L. reuteri* DSM 17938. In a recent survey, van Tilburg et al. interviewed more than 1000 American mothers in order to assess the prevalence of functional GI disorders in infants and toddlers. The prevalence of IC in children <1 year of age resulted 5.9%.[23] Conversely, in a recent review, some experts in the field hypothesized a likely prevalence of 20%, on the basis of the existing literature.[22] Despite the high prevalence and the elevated costs for national healthcare systems, the self-limiting nature of colic has limited the investigations to establish a pathophysiologic model of IC. Therefore, it is difficult to elucidate the mechanisms by which CAMs may act on symptoms' relief. The efficacy of the herbal formula could be partially explained by the hypothesis that IC may be related to a GI motor function disorder.[4] As a matter of fact, the studies of enteric neural control and maturation ontogeny of the stomach and of the small intestine support the concept that dysregulation and immaturity of intestinal motility contribute to the development of IC.[4] Already in 2005, Savino et al., in a double-blind RCT, demonstrated the efficacy of a herbal formula containing *Matricaria recutita* L., *F. vulgare* M. var. dulce and *M. officinalis* L. on 41 breastfed colicky infants.[15] Following clinical evidence, Capasso et al. conducted an in vivo study on a mouse model demonstrating that the administration of the herbal formula significantly modulated GI motility.[24] The effect on GI motility was mainly mediated by *Matricaria recutita* and *M. officinalis*. Indeed, it is well known that chamomilla (*Matricaria recutita*) has sedative[25,26] as well as antispasmodic and anti-inflammatory effects.[27–29] *Melissa officinalis* L. has both antispasmodic and sedative/anxiolytic activities.[30–32] The antispasmodic activity seems to be mainly effective on the GI smooth muscle, due both to the essential oil and flavonoid components, particularly apigenin and bisabololo.[30] Capasso et al. demonstrated that the pharmacological profile of the herbal formulation was superior over the single extracts in decreasing GI motility, hypothesizing possible synergisms among the constituents.[24] Taken together all these activities may partially demonstrate that herbal formulation are successful in the management of colicky infants trough a positive effect on GI motility. In the present study, *L. reuteri* DSM 17938 was superior to simethicone in the management of IC both in primary and secondary outcomes, confirming the results of previous RCTs.[10–12,33] Its efficacy was comparable to the standardized extract of *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122). The potential role of an altered intestinal microbiota in the pathogenesis of IC has recently been proposed to explain the efficacy of probiotics.[11] Savino et al. found that *Escherichia coli* were more abundant in the feces of colicky infants and speculated that coliform colonic fermentation and consequent excessive intra-intestinal air production may lead to aerophagia and pain.[34] More recently de Weerth et al. reported that infants with colic displayed lower microbiota diversity and stability compared with those without colic.[35] Probiotics may also directly modulate GI motility of infants with colics.[36,37] In addition, to the above reported herbs, the standardized extract tested in our study, is provided of tyndallized *L. acidophilus* (HA122). This probiotic has demonstrated in vitro anti-inflammatory and anti-infectious activities.[38–40] The process of tyndallization avoids the risks associated with the administration of live bacteria, leaving intact some soluble factors that can potentially exert some beneficial effects.[18] We speculate that the addition of tyndallized *L. acidophilus* (HA122) to the herbal formulation helps restoring a normal microbiota, contributing to colicky infants symptoms' relief. Indeed, Lactobacilli-secreted factors have been demonstrated to be a rich source of bacteriocins that restrict the growth and activities of different pathogens, to improve mucosal gut barrier integrity, and to modulate inflammatory mediators secretion.[18] Differently from previous studies conducted on *L. reuteri*,[10–12,33] which demonstrated an efficacy mainly in colicky breastfed infants, we did not find any difference between exclusively/partially breastfed infants and formula-fed infants in all the three groups of treatment. Although the sample of exclusively formula-fed infants was rather small, these findings may

suggest that the efficacy of the experimented drugs is independent of infants' feeding.

This study is not without limitations. Firstly, due to the differences of the three experimented products, we could not conduct a blinded RCT. The open-label design may have possibly influenced our results. Particularly, we cannot exclude that the efficacy of *M. chamomilla* L., *M. Officinalis* L. and tyndallized *L. acidophilus* (H122) may be partially explained by the common parents' belief that "natural means safe." In addition, the lack of a placebo group may have someway affected the outcomes of the study. Another significant limitation of the study, similar to previous studies on IC, is the absence of an objective way to assess the duration of crying and fussing times of infants, which fully relied on parents' reports.

5 | CONCLUSION

This study suggests that the administration of *M. chamomilla* L., *M. officinalis* L. and tyndallized *L. acidophilus* (HA122) and *L. reuteri* DSM 17938 are significantly more effective than simethicone in improving colic symptoms. Further, well-designed, double-blind RCT are needed to confirm this preliminary evaluation.

CONFLICTS OF INTEREST

Annamaria Staiano served as investigator and member of advisory board for the following companies: D.M.G, Valeas, Angelini, Miltè, Danone, Nestlé, Sucampo, and Menarini. Erasmo Miele served as speaker, as investigator and member of advisory board for the following companies: Abbvie, Angelini, Bioprojet, Ferring, Menarini, Milte, and Valeas. The remaining authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

MM, DU, FPG, and ES contributed to conception and design, analysis, and interpretation of data, drafting the article, and final approval of the version to be published; CT, DDG, IR, and SV were responsible for acquisition of data, drafting the article, and final approval of the version to be published; CR, AC, EM, and AS contributed to conception and design, revising the article critically for important intellectual content, and final approval of the version to be published; DB was responsible for analysis and interpretation of data, drafting the article and final approval of the version to be published. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

REFERENCES

1. Wake M, Morton-Allen E, Poulakis Z, Hiscock H, Gallagher S, Oberklaid F. Prevalence, stability, and outcomes of cry-fuss and sleep problems in the first 2 years of life: prospective community-based study. *Pediatrics*. 2006;117:836-842.
2. Lucassen PL, Assendelft WJ, van Eijk JT, Gubbels JW, Douwes AC, van Geldrop WJ. Systematic review of the occurrence of infantile colic in the community. *Arch Dis Child*. 2001;84:398-403.
3. Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. *Gastroenterology*. 2006;130:1519-1526.
4. Camilleri M, Park SY, Scarpato E, Staiano A. Exploring hypotheses and rationale for causes of infantile colic. *Neurogastroenterol Motil*. 2017;29. https://doi.org/10.1111/nmo.12943.
5. Petzoldt J, Wittchen HU, Wittich J, Einsle F, Höfler M, Martini J. Maternal anxiety disorders predict excessive infant crying: a prospective longitudinal study. *Arch Dis Child*. 2014;99:800-806.
6. Lee C, Barr RG, Catherine N, Wicks A. Age-related incidence of publicly reported shaken baby syndrome cases: is crying a trigger for shaking? *J Dev Behav Pediatr*. 2007;28:288-293.
7. Howard CR, Lanphear N, Lanphear BP, Eberly S, Lawrence RA. Parental responses to infant crying and colic: the effect on breastfeeding duration. *Breastfeed Med*. 2006;1:146-155.
8. Morris S, James-Roberts IS, Sleep J, Gillham P. Economic evaluation of strategies for managing crying and sleeping problems. *Arch Dis Child*. 2001;84:15-19.
9. Cohen-Silver J, Ratnapalan S. Management of infantile colic: a review. *Clin Pediatr*. 2009;48:14-17.
10. Savino F, Pelle E, Palumeri E, Oggero R, Miniero R. *Lactobacillus reuteri* (America Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. *Pediatrics*. 2007;119:e124-e130.
11. Savino F, Cordisco L, Tarasco V, et al. *Lactobacillus reuteri* DSM 17 938 in infantile colic: a randomized, double-blind, placebo-controlled trial. *Pediatrics*. 2010;126:e256-33.
12. Szajewska H, Gyrczuk E, Horvath A. *Lactobacillus reuteri* DSM 17938 for the management of infantile colic in breastfed infants: a randomized, double-blind, placebo-controlled trial. *J Pediatr*. 2013;162:257-262.
13. Sung V, Hiscock H, Tang ML, et al. Treating infant colic with the probiotic *Lactobacillus reuteri*: double blind, placebo controlled randomised trial. *BMJ*. 2014;348:2107.
14. Harb T, Matsuyama M, David M, Hill RJ. Infant colic-what works: a systematic review of interventions for breast-fed infants. *J Pediatr Gastroenterol Nutr*. 2016;62:668-686.
15. Perry R, Hunt K, Ernst E. Nutritional supplements and other complementary medicines for infantile colic: a systematic review. *Pediatrics*. 2011;127:720-733.
16. Savino F, Cresi F, Castagno E, Silvestro L, Oggero R. A randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis* (ColiMil) in the treatment of breastfed colicky infants. *Phytother Res*. 2005;19:335-340.
17. Tsilingiri K, Barbosa T, Penna G, et al. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. *Gut*. 2012;61:1007-1015.
18. Cicenia A, Scirocco A, Carabotti M, Pallotta L, Marignani M, Severi C. Postbiotic activities of lactobacilli-derived factors. *J Clin Gastroenterol*. 2014;48(Suppl 1):S18-S22.
19. Alves JG, de Brito Rde C, Cavalcanti TS. Effectiveness of *Mentha piperita* in the treatment of infantile colic: a crossover study. *Evid Based Complement Alternat Med*. 2012;2012:981352.
20. Barr RG, Kramer MS, Leduc DG, McVey-White L, Pless IB. Parental diary of infant cry and fuss behavior. *Arch Dis Child*. 1988;63:380-387.
21. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol*. 2004;159:702-706.
22. Vandenplas Y, Abkari A, Bellaiche M, et al. Prevalence and health outcomes of functional gastrointestinal symptoms in infants from birth to 12 months of age. *J Pediatr Gastroenterol Nutr*. 2015;61:531-537.
23. van Tilburg MA, Hyman PE, Walker L, et al. Prevalence of functional gastrointestinal disorders in infants and toddlers. *J Pediatr*. 2015;166:684-689.

24. Capasso R, Savino F, Capasso F. Effects of the herbal formulation ColiMil on upper gastrointestinal transit in mice in vivo. *Phytother Res*. 2007;21:999-1101.
25. Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (*Matricaria chamomilla* L.): an overview. *Pharmacogn Rev*. 2011;5:82-95.
26. Amsterdam JD, Li Y, Soeller I, Rockwell K, Mao JJ, Shults J. A randomized, double-blind, placebo-controlled trial of oral *Matricaria recutita* (chamomile) extract therapy for generalized anxiety disorder. *J Clin Psychopharmacol*. 2009;29:378-382.
27. Dell'Agli M, Di Lorenzo C, Badea M, et al. Plant food supplements with anti-inflammatory properties: a systematic review. *Crit Rev Food Sci Nutr*. 2013;53:403-413.
28. Maschi O, Cero ED, Galli GV, Caruso D, Bosisio E, Dell'Agli M. Inhibition of human cAMP-phosphodiesterase as a mechanism of the spasmolytic effect of *Matricaria recutita* L. *J Agric Food Chem*. 2008;56:5015-5020.
29. Mehmood MH, Munir S, Khalid UA, Asrar M, Gilani AH. Antidiarrhoeal, antisecretory and antispasmodic activities of *Matricaria chamomilla* are mediated predominantly through K(+)-channels activation. *BMC Complement Altern Med*. 2015;15:75.
30. Soulimani R, Younas C, Fleurentin J, Mortier F, Misslin R, Derrieux G. Biological activity study of *Melissa officinalis* on central nervous system by in vivo mouse and duodenum of in vitro rat. *Plant Med Phytother*. 1993;26:77-85.
31. Feliú-Hemmelmann K, Monsalve F, Rivera C. *Melissa officinalis* and *Passiflora caerulea* infusion as physiological stress decreaser. *Int J Clin Exp Med*. 2013;6:444-451.
32. Hajhashemi V, Safaei A. Hypnotic effect of *Coriandrum sativum*, *Ziziphus jujuba*, *Lavandula angustifolia* and *Melissa officinalis* extracts in mice. *Res Pharm Sci*. 2015;10:477-484.
33. Chau K, Lau E, Greenberg S, et al. Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating *Lactobacillus reuteri* DSM 17938. *J Pediatr*. 2015;166:74-78.
34. Savino F, Cordisco L, Tarasco V, Calabrese R, Palumeri E, Matteuzzi D. Molecular identification of coliform bacteria from colicky breastfed infants. *Acta Paediatr*. 2009;98:1582-1588.
35. de Weerth C, Fuentes S, Puylaert P, de Vos WM. Intestinal microbiota of infants with colic: development and specific signatures. *Pediatrics*. 2013;131:e550-e558.
36. Kannampalli P, Pochiraju S, Chichlowski M, et al. Probiotic *Lactobacillus rhamnosus* GG (LGG) and prebiotic prevent neonatal inflammation-induced visceral hypersensitivity in adult rats. *Neurogastroenterol Motil*. 2014;26:1694-1704.
37. Wu RY, Pasyk M, Wang B, et al. Spatiotemporal maps reveal regional differences in the effects on gut motility for *Lactobacillus reuteri* and rhamnosus strains. *Neurogastroenterol Motil*. 2013;25:e205-e214.
38. Liévin-Le Moal V. A gastrointestinal anti-infectious biotherapeutic agent: the heat-treated Lactobacillus LB. *Therap Adv Gastroenterol*. 2016;9:57-75.
39. Huang IF, Lin IC, Liu PF, et al. *Lactobacillus acidophilus* attenuates Salmonella-induced intestinal inflammation via TGF-β signaling. *BMC Microbiol*. 2015;15:203.
40. Patman G. Gut microbiota: *Lactobacillus acidophilus* opens the door to butyrate. *Nat Rev Gastroenterol Hepatol*. 2015;12:552.

How to cite this article: Martinelli M, Ummarino D, Giugliano FP, et al. Efficacy of a standardized extract of *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *Lactobacillus acidophilus* (HA122) in infantile colic: An open randomized controlled trial. *Neurogastroenterol Motil*. 2017;e13145. https://doi.org/10.1111/nmo.13145