Vol.67: e24230951, 2024
https://doi.org/10.1590/1678-4324-2024230951
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Human and Animal Health

Emulgels Containing Propolis and Curcumin for the Treatment of Mastitis and Umbilical Cord Healing

Rafaela Said dos Santos[1]
https://orcid.org/0000-0002-6473-4869

Katieli da Silva Souza Campanholi[2]
https://orcid.org/ 0000-0001-5156-8542

Amanda Sandes Bispo[3]
https://orcid.org/0000-0002-0655-4093

Wilker Caetano[2]
https://orcid.org/ 0000-0002-9402-8324

Magali Soares dos Santos Pozza[3]
https://orcid.org/0000-0002-3500-1914

Henrique Leal Perez[3]
https://orcid.org/ 0000-0003-0630-8287

Marcos Luciano Bruschi[1]*
https://orcid.org/ 0000-0002-4838-5742

[1]Universidade Estadual de Maringá, Departamento de Farmácia, Maringá, Paraná, Brasil; [2]Universidade Estadual de Maringá, Departamento de Química, Maringá, Paraná, Brasil; [3]Universidade Estadual de Maringá, Departamento de Zootecnia, Maringa, Paraná, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 10-Sep-2023; Accepted: 25-Jan-2024

*Correspondence: mlbruschi@uem.br; Tel.: +55-44-3011-5998 (M.L.B.)

HIGHLIGHTS

- Curcumin-propolis emulgels for the treatment of mastitis and umbilical cord healing.
- The emulgels showed good healing activity on lamb navels.
- The systems prevented mastitis in dairy cows.
- Formulations showed promising antimicrobial and healing activities in animals.

Abstract: Brazil is a large sheep and cow producer, and the development of new strategies for the treatment of lamb umbilical cord healing as well as the bovine mastitis is very important. The aim of this study was to develop emulgels containing propolis, curcumin and andiroba oil for the treatment of mastitis and umbilical cord healing in animals. The formulations were composed of poly (acrylic acid) derivative (Carbopol 974P or polycarbophil), Brazilian green propolis extract, curcumin, and andiroba oil. The systems were investigated as the activity on umbilical cord healing of newborn lambs, and evaluation of the prevention of mastitis in dairy cows. The emulgels effectively promoted umbilical cord healing, preventing infections. Moreover, they were effective for reducing the somatic cell count a health indicator for mammary glands in cows. These findings suggest that these formulations can potentially treat cattle mastitis and improve lambs' umbilical cord healing.

Keywords: emulsion system; natural products; mastitis; antimicrobial; healing.

INTRODUCTION

Brazil is a large sheep producer, with an effective at 8.7 million head, 90% of which are in the Northeast region [1]. The umbilical cord serves as a channel for the supply of blood and nutrients throughout pregnancy. When the cord breaks during the birthing process, it leaves an umbilical stump that becomes a risk because it becomes an entrance for pathogens in the newborn, increasing the risk of infections [2]. In this context, antiseptic compounds help clean, sanitize, and speed healing the umbilical stump. The two most common choices of antiseptic compounds are 7% iodine or 4% chlorhexidine. However, the antiseptic properties begin to diminish approximately 15 minutes after application, increasing interest in exploring the effectiveness of new treatment alternatives [2].

Moreover, bovine mastitis is a significant disease because of the economic losses to dairy producers. This disease is characterized by a mammary gland inflammatory response caused by metabolic and physiologic changes, trauma or, more frequently, contagious, or environmental pathogenic microorganisms [3]. Costs due to mastitis include reduced milk production, condemnation of milk due to antibiotic residues, veterinary costs, culling of chronically infected cows, and occasional death mastitis has a serious zoonotic potential associated with the shedding of bacteria and their toxins in the milk [4,5]; *Staphylococcus aureus* bacteria are frequently isolated in addition to *Streptococcus dysgalactiae, Streptococcus agalactiae, and Streptococcus uberis* [6].

The prevalence of mastitis in dairy herds has resulted in the extensive use of antibiotics [7]. However, treatment with antibiotics during lactation has limited efficacy and leads to increased residues found in commercial milk samples [8].

Main changes in the udder includes leakage of ions, proteins, and enzymes into the milk due to increased vascular permeability, decreased synthesis of caseins and lactose, and invasion of phagocytizing cells into the milk compartment [9]. The main changes are increased sodium, chloride, and serum protein levels and reduced calcium, lactose, and casein [10].

Measuring the somatic cells count (SCC) in milk is the gold standard for ruling out the severity of mastitis. Usually, in milk from a healthy mammary gland, the SCC is lower than 10^5 cells/ mL, while bacterial infection can cause it to increase to above 10^6 cells/mL [11]. Despite intensive research and implementation of various mastitis control strategies over the last few decades, bovine mastitis has not yet disappeared and is insurmountable in dairy profitability [12].

The study of new therapeutic agents for mastitis in cattle should begin with screening a potential product for its antimicrobial effects on the principal etiological agents causing the disease. For mastitis, the search for an antimicrobial product with a broad spectrum of biological properties is directly related to the fact that an inflammatory response accompanies the bacterial infection. As such, therapeutic agents with anti-inflammatory, antioxidant, and immunostimulatory properties are needed to improve the overall health of the mammary gland and the animal [13].

The use of multiple antioxidant and healing chemotherapeutic agents may favor the treatments. Propolis (PRP) is a complex mixture of plant substances. It has a wide range of biological activities attributed to its chemical constituents, which are mainly phenolic acids and polyphenols, such as flavonoids [14]. Many biological activities have been described for propolis, such as antioxidant, antimicrobial, antiproliferative, antitumor, anti-inflammatory, and immunomodulatory [15,16]. Curcumin (CUR), present in saffron, a common spice from India and China, is a widely studied bioactive agent for the treatment and prevention of various diseases, such as cystic fibrosis, psoriasis, depression, asthma, arthritis, brain damage, diabetes, and healing. It exhibits anti-inflammatory, antioxidant, and antimicrobial action and can help to prevent cardiovascular diseases [17-20].

Moreover, semi-solid systems are frequently utilized as controlled drug delivery for topical route of administration, enabling the increase of residence time and of the bioavailability of different active agents [14]. Emulsion is defined as coarse or colloidal dispersion that can show important properties for topical drug delivery: easy for spreading, improved skin permeation, not greasy, water soluble, emollient, pleasant appearance, good rheology and long shelf life. The combination of emulsions and gels results in emulgels, which are emulsion systems gelled by mixing polymeric gelling agent and aqueous phase thickener. This strategy also enables the obtention of more stable emulsion systems due to the interfacial tension decrease and the aqueous-phase viscosity increase [14,21]. They can incorporate well both hydrophilic and hydrophobic drugs, offer new drug delivery platforms, facilitate administration, improve retention, permeation, absorption, and probably control the release of EPRP and/or CUR [22, 23]. In addition, they are systems that require an oil phase that will perform an emollient/moisturizing action, promoting skin hydration. For this phase, andiroba oil was chosen, widely used to treat skin conditions and muscle inflammation, and used as a natural insect repellent [24].

In this work, emulgels containing the two active agents (PRP and CUR), along with andiroba oil, were utilized for the tests on the umbilical healing of lambs and the evaluation of mastitis prevention in dairy cows.

MATERIAL AND METHODS

Materials

Carbopol 974P® (C974P) and polycarbophil (PC) were received from Lubrizol (Sao Paulo, SP, Brazil). Curcumin C3 complex® was received from Sabinsa® (West Windsor, USA) and triethanolamine, used as a neutralizing agent, was purchased from Galena (Campinas, SP, Brazil). Andiroba oil (AO) was obtained from the mechanical oil extraction process upon selected seeds from rainforest Andiroba tress (*Carapa guianensis*) in the Tome-Açu Agroextractive Association (Quatro Bocas-Tome-Acu, Para State, Brazil, under the geographic DMS coordinates of -2° 24' 59.99" S latitude, -48° 08' 60.00" W longitude). Forest management was conducted considering the sustainability and the conservation of ecosystem and Amazonian biodiversity. Only the seeds fallen from the *Carapa guianensis* tress, collected in the whole oriental amazon rainforest region in a sustainable way, were submitted to pure AO extraction clean process. All the processes following the environmental legislation in force through the National System for Management of Genetic Heritage (SISGEN nº. A098049), as well as duly authorized by the same association. Brazilian green propolis (PRP) was obtained from an apiary (of *Apis mellifera* L. bees) in the northwest of Parana state (SISGEN nº. AC7A2F5). Purified water was obtained in-house using a water purification system (Evoqua Water Technologies, Pittsburgh, USA).

Preparation of systems

The optimized emulgels were prepared using C974P or PC at a concentration of 1.0% (w/w) [14]. The polymer dispersion was carried out in purified water under mechanical agitation at 200 rpm and at room temperature. After complete dispersion, CUR (0.1%, w/w) was added under constant stirring, and the pH adjusted to 7.0 using triethanolamine [25, 26]. Propolis extract (EPRP) (8%, w/w) was added to the systems gradually, and finally, AO (8%, w/w) was incorporated, maintaining constant mechanical stirring, according to Table 1. After preparation, all formulations were stored in hermetically sealed bottles for at least 24 h before characterization analyses.

Table 1. Composition of emulgel systems composed of different poly (acrylic acid derivatives [Carbopol 974P (C974P) or polycarbophil (PC)], curcumin (CUR), andiroba oil (AO), Brazilian green propolis extract (EPRP).

Formulation	Polymer type	AO (%, w/w)	Bioactive agents (%, w/w)	
			EPRP	CUR
F1	C974	8.0	8.0	0.1
F2	PC	8.0	8.0	0.1

Analysis of activity on umbilical cord healing of newborn lambs

The newborn lambs were identified and distributed in an entirely randomized design (DIC), treated in three different groups: seven animals treated with iodine (control/standard treatment), eight animals treated with formulation F1, and eight animals treated with formulation F2, for three days, once a day. After birth, the treatments were applied, and then the lambs were released into open pasture. For two more days, the animals were captured, and the treatments were applied again. To control infection, the rectal temperature of the animals was monitored for three days, and on the 5th day, blood tests were performed on three animals in each group to analyze the leukocyte cells. This test was approved by the Ethics Committee on Animal Studies of UEM (Authorization N°. 3425280722), and due to the accepted norms for minimum care, no animal was left untreated [27].

For leukocyte cell analysis, approximately 5 mL of blood sample was collected with the help of 40 x 1.20 mm needles. This collected blood was transferred to test tubes with an anticoagulant (ethylenediaminetetraacetic acid - EDTA). The analysis of these cells was performed by a veterinary clinical analysis laboratory (NAV - *Núcleo de apoio Veterinário*), in the city of Maringa, PR, Brazil. All analyses were performed at least in three replicate samples.

Evaluation of the applicability on treatment of mastitis in dairy cows

The experiment was conducted in the cattle sector of the Experimental Farm of Iguatemi – State University of Maringa (FEI) under approval by the Animal Ethics Committee of the State University of Maringa - CEUA, process nº 3425280722. Holstein and Jersey cows were used, with an average weight of 500 kg and average production of 20 liters/day, in different stages of lactation (beginning, peak and end), kept in pasture and supplemented with corn silage and concentrate. The experiment was carried out in a cross-over design being post-dipping applications: T1 (control treatment using lactic acid; Ekomilk after gel film, Alto da Pedra Branca, Brazil) and T2 (formulation F2). A total of six animals were utilized for each experiment/treatment.

Milk samples were collected at 0, 7, and 14 days of product application for somatic cell count (SCC) and physicochemical composition. The somatic cell count and physicochemical composition of cow's milk were performed in the milk analysis laboratory Mesoregional Center of Excellence in Milk Technology and were determined by the ultrasonic milk analyzer Ekomilk Scan and Master Classic, respectively. All analyses were performed at least in three replicate samples.

The data obtained in vitro and in situ were subjected to analysis of variance (ANOVA), and the significant difference between the means ($p < 0.05$) was determined by Tukey's test using SAS 9.3 software (Statistical Analysis System Institute, Cary, NC).

RESULTS

Emulgels (F1 and F2) containing CUR, EPRP and OA displayed good appearance and consistency (Figure 1), according previous studies [14,28].

Figure 1. Macroscopic characteristics of the emulgel systems F1 and F2.

Activity on umbilical cord healing of newborn lambs

Within the 23 animals analyzed, two died, and one presented myiasis in the navel. Except for these three animals, the rest responded well to the healing treatment (Figure 2).

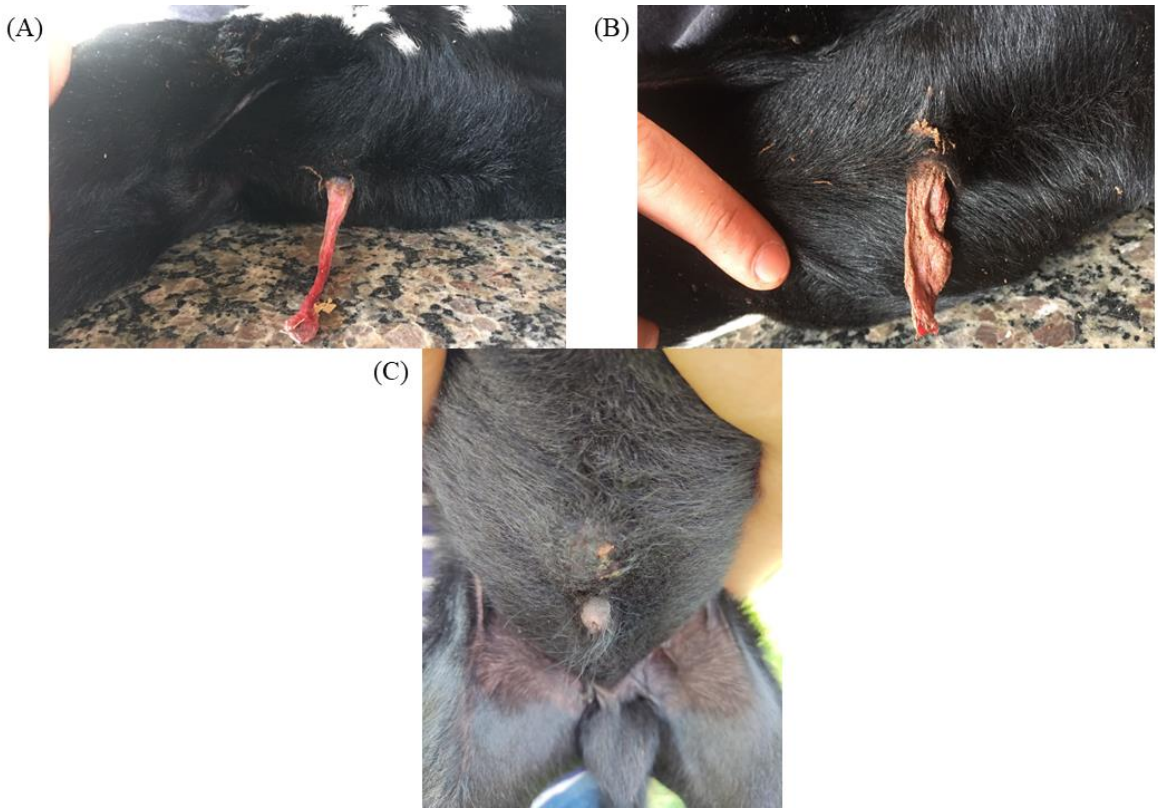

Figure 2. Photographs showing the lamb navel treated using emulgel F2: (A) newborn; (B) after application of the formulation F2; (C) after treatment and complete healing.

The rectal temperature of the evaluated animals varied from 36.8 ºC to 39.4 °C, with no indication of febrile cases. Besides this parameter, blood tests were performed on the animals, where the leukocyte cells were evaluated (Figure 3). The reference values for this test range from 4,000 to 12,000 µL.

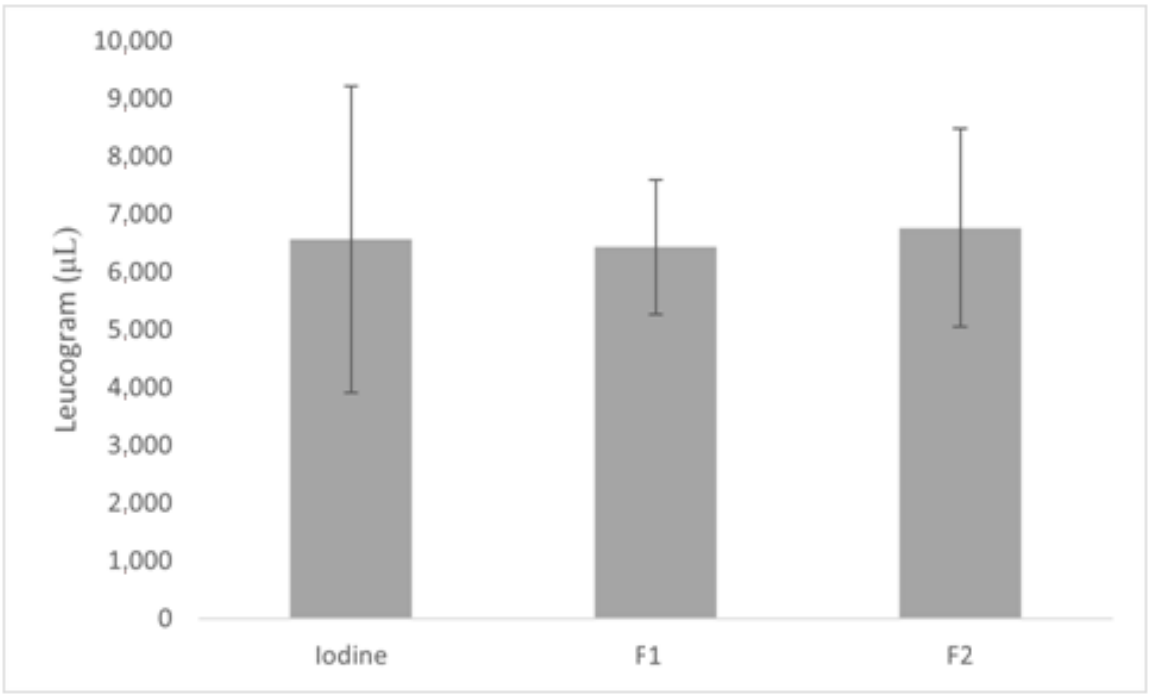

Figure 3. Evaluation of infectious process of the average of three animals of each treatment (iodine, F1 and F2).

All groups, with the different treatments presented leukogram results within the reference range, and in no animal was an increase in the number of leukocytes observed.

Evaluation of the applicability on treatment of mastitis in dairy cows

For this study, the F2 system was chosen and the Table 2 displays the results of the analyzed data.

Table 2. Analysis of the treatment with the emulgel F2 and the control treatment (lactic acid) on the different days analyzed for the different parameters.

Parameters	Treatments (mean)		*p-valor*			
	Control	F2	P_{trat}	P_{day}	P_{txd}	EPM
SCC (10^3 cells/mL)	500.600	317.300	0.066	0.080	0.763	0.066
G (%)	4.71	4.57	0.677	0.004	0.772	0.172
ST (%)	9.88	9.85	0.835	0.250	0.547	0.064
D (g/mL)	1.03	1.03	0.971	0.651	0.324	<0.001
P (%)	3.65	3.63	0.700	0.253	0.416	0.024
SL (%)	0.83	0.83	0.658	0.163	0.673	0.006
pH	6.86 ± 0.51	6.78 ± 0.68	0.660	0.005	0.931	0.099

Averages of treatments with different lowercase letters in the same row are significantly different for days ($p < 0.05$). P_{trat} - treatment effect; P_{day} - days effect; P_{txd} - interaction between treatment and days; EPM - Standard Error of the Means; G= fat, ST= total solids, D= density, P= protein, SL= salts.

DISCUSSION

In previous studies [14,28], we have developed and selected the emulgels F1 and F2, due to their promising characteristics (e.g., antioxidant and antimicrobial activity, enabled modified/prolonged drug release), for further investigations as topical delivery systems of CUR and EPRP. Moreover, they were chosen because they were more stable and displayed good appearance and consistency, with suitable mechanical and physicochemical properties. F1 and F2 had in common the bioactive compounds (EPRP+CUR) and OA, responsible for better structuring of the systems, as well as increasing some texture parameters, such as adhesiveness [14,28], which means that they are difficult to break and remain in contact with the application site for longer, justifying their use in animal tests.

Therefore, in this study, we have evaluated these two selected emulgels as the capacity of promote umbilical healing of lambs and the mastitis prevention in dairy cows.

The umbilical cord is a placenta structure that, after birth, becomes an entry site for pathogens, causing diseases like myiasis, which is defined as a lesion caused by the larvae of the fly *Cochliomyia hominivorax* (varejeira). It lay many eggs, around 200-300 units, around wounds or the navel of newborns. These eggs hatch, and the larvae migrate into the wound, creating a cavity inside the wound, progressively increasing in depth. The damage caused is severe and can lead the animal to death [29]. Umbilical infections are a health risk for animals. Studies indicate that up to 20% of dairy calves develop umbilical infections [27,30,31] and 1.6% of reported calf deaths are related to umbilical infections [2,27]. In this regard, using systems with specific drugs to prevent these diseases is essential.

Typically, antiseptic compounds help clean, sanitize, and improve the rate of umbilical healing while reducing the risk of infection to the animal. The two compounds commonly used for treatment are 7% iodine and chlorhexidine [27,31]. Emulgel formulations were chosen for the prevention of these diseases because, in addition to the excellent healing action of propolis extract, as described by Burdock (1998) and De Francisco (2013) [32,33], these systems also contain CUR, which has excellent anti-inflammatory action [17,34,35], as well as AO, which is known as an insect repellent [36,37].

The animals responded well to both healing treatments (iodine, F1 and F2) and no fever was observed [37,38]. The results were similar, but it was possible to observe the formulation F2 displayed better performance (Figure 2). The alcoholic solution of iodine (7%) was chosen as the standard treatment due to it can kill most pathogens with a short contact time, and the ethanol presence can help in an increased umbilical stump desiccation rate [27]. However, the problems for local administration of this solution (e.g. hurts due to the ethanol presence and low rheological properties), difficult about storage and increased

regulatory concerns, justify the use of emulgels, mainly F2. The leukogram results indicated the animals did not present any infectious condition and confirming that the treatments were effective.

Moreover, epidemiological studies on risk factors have identified characteristics related to the environment, management procedures, and milking equipment associated with bovine mastitis and variation in SCC [39,40]. To ensure milk quality, producers must ensure that the product has the characteristics established in Normative Instruction 76 (IN 76) regarding SCC, total solids, fat, protein, lactose and minerals, taste, odor, and appearance [41].

For the investigation of the applicability on treatment of mastitis in dairy cows, the emulgel F2 was selected due to the PC polymer, which is made up of numerous hydrophilic groups such as amine, carboxyl, and hydroxyl groups. This is called the degree of swelling, which is directly proportional to the amount of water absorbed by the emulgel [42], making it easier to release the active ingredients.

In the present study, both treatments displayed similar efficiency (safety). The statistical comparison between F2 and the control treatment using lactic acid displayed no significant difference ($p > 0.05$) for all parameters (Table 2). However, considering the effect of days, the fat content displayed significant difference between the two treatments ($p < 0.05$). Variations in protein, fat and lactose levels in milk can be caused by diet, health, milking management, season, lactation phase, genetics, and breed [43]. The increase in fat levels during collection days probably refers to lactation stages or diet.

For the safety and quality of milk, the recommended SCC value is 500,000 cells/mL (IN 76/2018). High SCC scores are associated with changes in milk composition, resulting in poor quality of milk and dairy products. SCCs in milk are influenced by many factors, such as animal species, milk production level, lactation stage, and the individual and environmental factors in addition to management practices. A threshold of ≤ 200,000 cells/mL is the most practical value for determining breast quarter health [12]. The treatment using F2 displayed a lower value for SCC than using lactic acid.

Previous studies showed the effectiveness of propolis in the treatment of bovine mastitis. The use and efficiency of this natural compound as a pre- and post-dip has also been verified previously. Fiordalisi (2015) found that propolis has the potential to be used to treat subclinical mastitis or during dry cow therapy when the bacterial count is comparable to the clinical condition [13]. Schelles and colleagues (2021) found no difference between treatments on milk protein, lactose and fat concentration when evaluating the use of propolis and iodine as pre- and post-soaking for lactating cows for 28 days [44]. Melo Peixoto (2012) observed the efficacy of propolis as post-immersion and administered orally to cows, when compared to a commercial product [45]. Thus, emulgel F2 showed to be an alternative product for lactic acid gel film for the treatment of mastitis.

CONCLUSION

The optimized emulgels containing propolis, curcumin and andiroba were in vivo evaluated as their activity on umbilical healing of lambs and mastitis prevention in dairy cows. These formulations have good appearance and consistency, suitable for topical administration. Both emulgels F1 and F2 were efficient for umbilical healing and similar to iodine alcoholic solution 7%. Furthermore, emulgel F2 had the same efficiency as the commercial product (lactic acid gel). The F2 system proved to be a promising alternative formulation for the treatment of these diseases and constitutes a candidate for further in vivo veterinary tests.

Acknowledgments: This research was supported by the Brazilian agencies CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico/National Council for Scientific and Technological Development; Processes nº 307695/2020-4 and nº. 404163/2021-1), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Coordination for the Improvement of Higher Education Personnel; Finance Code 001), and FINEP (Financiadora de Estudos e Projetos /Financier of Studies and Projects of Brazil) and INCT/milk.
Conflict of interest: The authors declare no conflict of interest.

REFERENCES

1. Da Silva FLR, de Figueiredo EAP, Simplício AA, Barbieri ME, Arruda F de A V. [Genetic and phenotypic parameters for the weights of native and exotic goats, raised in northeastern Brazil, in the growth phase]. Rev da Soc Bras Zootec. 1993;22(2):350–9.
2. Robinson AL, Timms LL, Stalder KJ, Tyler HD. The effect of four antiseptic compounds on umbilical cord healing and infection rates in the first 24 h in dairy calves from a commercial herd. J Dairy Sci. 2015;98:5726–8.
3. Oviedo-Boyso J, Valdez-Alarcón JJ, Cajero-Juárez M, Ochoa-Zarzosa A, López-Meza JE, Bravo-Patiño A, et al. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. J Infect. 2007;54(4):399–409.

4. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. 2003;34:475–91.
5. González RN, Wilson DJ. Mycoplasmal mastitis in dairy herds. Vet Clin North Am - Food Anim Pract. 2003;19(1):199–221.
6. Soltau JB, Einax E, Klengel K, Katholm J, Failing K, Wehrend A, et al. Within-herd prevalence thresholds for herd-level detection of mastitis pathogens using multiplex real-time PCR in bulk tank milk samples. J Dairy Sci [Internet]. 2017;100(10):8287–95. Available from: http://dx.doi.org/10.3168/jds.2016-12385
7. Bradley AJ. Bovine mastitis: An evolving disease. Vet J. 2002;164(2):116–28.
8. Mukherjee R, Dash PK, Ram GC. Immunotherapeutic potential of Ocimum sanctum (L) in bovine subclinical mastitis. Res Vet Sci. 2005;79(1):37–43.
9. Østerås O, Edge VL, Martin SW. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J Dairy Sci. 1999;82(6):1221–31.
10. Kitchen BJ. Bovine mastitis: Milk compositional changes and related diagnostic tests. J Dairy Res. 1981;48(1):167–88.
11. Bytyqi H, Zaugg U, Sherififi K, Hamidi A, Gjonbalaj M, Muji S, et al. Influence of management and physiological factors on somatic cell count in raw cow milk in Kosova. Vet Arh. 2010;80(2):173–83.
12. Malik TA, Mohini M, Mir SH, Ganaie BA, Singh D, Varun TK, et al. Somatic Cells in Relation to Udder Health and Milk Quality-A Review. J Anim Heal Prod. 2018.
13. Fiordalisi SAL, Honorato LA, Loiko MR, Avancini CAM, Veleirinho MBR, Filho LCPM, et al. The effects of Brazilian propolis on etiological agents of mastitis and the viability of bovine mammary gland explants. J Dairy Sci [Internet]. 2016;99(3):2308–18. Available from: http://dx.doi.org/10.3168/jds.2015-9777
14. Said dos Santos R, Bassi da Silva J, Rosseto HC, Vecchi CF, Campanholi K da SS, Caetano W, et al. Emulgels Containing Propolis and Curcumin: The Effect of Type of Vegetable Oil, Poly(Acrylic Acid) and Bioactive Agent on Physicochemical Stability, Mechanical and Rheological Properties. Gels. 2021 Aug;7(3):120.
15. Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini AG, et al. Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochem Anal. 2004;15(4):235–40.
16. Bruschi ML, Klein T, Lopes RS, Franco SL, Gremião MPD. Contribution to the quality control protocol of propolis and its extracts. Rev Ciencias Farm. 2002;23(2):289–306.
17. Sueth-Santiago V, Mendes-Silva GP, Decoté-Ricardo D, Lima MEF de. Curcumin, the golden powder from turmeric: insights into chemical and biological activities. Quim Nova. 2015;38(4):538–52.
18. Goel A, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. Nutr Cancer. 2010;62(7):919–30.
19. Ji H-F, Shen L. Can improving bioavailability improve the bioactivity of curcumin? Trends Pharmacol Sci. 2014;35(6):265–6.
20. Mazzarino L, Travelet C, Ortega-Murillo S, Otsuka I, Pignot-Paintrand I, Lemos-Senna E, et al. Elaboration of chitosan-coated nanoparticles loaded with curcumin for mucoadhesive applications. J Colloid Interface Sci. 2012;370(1):58–66.
21. Singla V, Saini S, Joshi B, Rana AC. Emulgel: A new platform for topical drug delivery. Int J Pharma Bio Sci. 2012;3(1):485–98.
22. Jones DS, Bruschi ML, de Freitas O, Gremião MPD, Lara EHG, Andrews GP. Rheological, mechanical and mucoadhesive properties of thermoresponsive, bioadhesive binary mixtures composed of poloxamer 407 and carbopol 974P designed as platforms for implantable drug delivery systems for use in the oral cavity. Int J Pharm. 2009;372(1–2).
23. De Souza Ferreira SB, Moço TD, Borghi-Pangoni FB, Junqueira MV, Bruschi ML. Rheological, mucoadhesive and textural properties of thermoresponsive polymer blends for biomedical applications. J Mech Behav Biomed Mater. 2015;65:164–78.
24. Souza JO de, Freire ACS, Albuquerque PM, Silva GF da. [Thermodynamic and kinetic study of emulsions containing andiroba oil (*Carapa guianensis*)]. An do Congr Bras Eng Química. 2016;1.
25. Bruschi ML. [Development and characterisation of into the peridontal pocket propolis delivery systems]. Faculdade de Ciências Farmacêuticas, Universidade de São Paulo; 2006.
26. De Araújo Pereira RR, Bruschi ML. Vaginal mucoadhesive drug delivery systems. Drug Development and Industrial Pharmacy. 2012.
27. Fordyce AL, Timms LL, Stalder KJ, Tyler HD. Short communication: The effect of novel antiseptic compounds on umbilical cord healing and incidence of infection in dairy calves. J Dairy Sci [Internet]. 2018;101(6):5444–8. Available from: http://dx.doi.org/10.3168/jds.2017-13181
28. Said dos Santos R, Bassi da Silva J, Vecchi CF, Campanholi KDSS, Rosseto HC, Oliveira MC de, et al. Formulation and performance evaluation of emulgel platform for combined skin delivery of curcumin and propolis. Pharm Dev Technol. 2023;28(6):559–70.
29. Retore M, Correa EB. [Main diseases diagnosed in sheep herds in Mato Grosso do Sul]. Embrapa Agropecuária Oeste. 2015;1:25–34.
30. Virtala AMK, Mechor GD, Grohn YT, Erb HN. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York state. J Dairy Sci. 1996;79:1040–9.
31. Mee JF. Managing the calf at calving time. Am Assoc Bov Pr Proc. 2008;41:46–53.

32. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol. 1998;36(4):347–63.
33. De Francisco LMB, Cerquetani JA, Bruschi ML. Development and characterization of gelatin and ethylcellulose microparticles designed as platforms to delivery fluoride. Drug Dev Ind Pharm. 2013;39(11):1644–50.
34. Goel A, Aggarwal BB. Curcumin, the Golden Spice From Indian Saffron, Is a Chemosensitizer and Radiosensitizer for Tumors and Chemoprotector and Radioprotector for Normal Organs. Nutr Cancer. 2010 Sep;62(7):919–30.
35. Gupta SC, Patchva S, Koh W, Aggarwal BB. Discovery of Curcumin, a Component of the Golden Spice, and Its Miraculous Biological Activities. Clin Exp Pharmacol Physiol [Internet]. 2012;39(3):283–99. Available from: http://digital.csic.es/bitstream/10261/64260/1/Biosíntesis de penicilina.pdf
36. Cela EVSS, da Rocha Mã de B, Gomes TM, Chia CY, Alves CF. Clinical evaluation of the effectiveness of andiroba oil in burns caused by hair removal with intense pulsed light: A prospective, comparative and double-blind study. Surg Cosmet Dermatol. 2012;4(3).
37. Brion A. [Veterinarian Vademecum]. 2 ed. Barcelona: Gea; 1964. 732 p.
38. Oliveira FMM, Dantas RT, Furtado DA. Parameters of thermal and physiological comfort of Santa Inês sheep in different conditioning systems. Eng Agrícola. 2005;9(4):631–5.
39. Souza GN, Brito JRF, Moreira EC, Brito MAVP, Silva MVGB. [Variation in somatic cell counts in dairy cows according to mastitis pathogens]. Arq Bras Med Vet e Zootec. 2009;61(5):1015–20.
40. Coentrão CM, Souza GN, Brito JRF, Paiva E Brito MAV, Lilenbaum W. Risk factors for subclinical mastitis in dairy cows. Arq Bras Med Vet e Zootec. 2008;60(2):283–8.
41. Brasil M da AP e A. Instrução normativa nº 76, de 26 de novembro de 2018. [Technical regulations that establish the identity and quality characteristics that refrigerated raw milk, pasteurized milk and type A pasteurized milk must present]. Diário Of da União, Brasília. 2018;
42. Do Nascimento MHM, Lombello CB. [Hyaluronic acid and chitosan based hydrogels for cartilage tissue engeneering];26(4):360–70.
43. Santos JHA, Guerios EM. [Main factors that influence the concentration of total solids in the milk of bovine females]. Arq Bras Med Veterinária. 2020;3(1):81–8.
44. Schelles JL, Rodrigues BM, dos Santos Pozza MS, de Lima LS. Use of propolis extract as antisseptic agent for pre and post dipping on dairy cows. Agrarian. 2021;14(51):95-101.
45. Heinzen EL, Peixoto ECT de M., Jardim JG, Garcia RC, de Oliveira NTE, de Oliveira Orsi R. Extract of propolis in the control of helminthiasis in calves. Acta Vet Bras. 2012;6(1):40–4.