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Potential of a phytotherapeutic emulgel composed of *Stryphnodendron adstringens* (Mart.) Coville and *Copaifera martii* for topical wound healing[1]

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ABSTRACT.- Silva Júnior R.C., Pelissari K.P., Morais F.A.P., Scapim M.R.S., Valenciano F.G., Perez H.L., Bezerra R.A.D., Pozza M.S.S., Caetano W. & Campanholi K.S.S. 2024. **Potential of a phytotherapeutic emulgel composed of *Stryphnodendron adstringens* (Mart.) Coville and *Copaifera martii* for topical wound healing.** *Pesquisa Veterinária Brasileira 44:e07354, 2024*. Departamento de Química, Núcleo de Pesquisas em Sistemas Fotodinâmicos e Nanomedicina, Universidade Estadual de Maringá, Av. Colombo 5790, Maringá, PR 87020-900, Brazil. E-mail: katieli_souza@hotmail.com

Myiasis is a worsening parasitic condition in newborn sheep, requiring effective medications for healing. This article demonstrates the development of a phytotherapeutic emulgel (phytogel) composed of copaiba oil (*Copaifera martii*) and "barbatimão" (*Stryphnodendron adstringens* (Mart.) Coville) incorporated into a polymeric blend composed of Pluronic® F127 and Carbopol® 934P. The formulation demonstrated antimicrobial potential by inactivating the bacteria *Staphylococcus aureus*. Administering the phytotherapeutic to the umbilicus of newborn lambs for five days effectively promoted healing and prevented inflammation and myiasis. The erythrogram and leukogram of the animals showed statistical equivalence between the animals treated with the phytotherapeutic and iodine. Furthermore, the human intervention index of the phytogel was lower when compared to iodine. The results underscore the medication's potential, warranting further studies in veterinary medicine.

INDEX TERMS: Myiasis, copaiba oil, barbatimão, wounds, navel healing, sheep.

RESUMO.- [**Potencial de emulgel fitoterapêutico composto por *Stryphnodendron adstringens* (Mart.) Coville e *Copaifera martii* para cicatrização tópica de feridas.**] Miíase é um quadro de parasitismo agravante em ovinos recém-nascidos, requerendo medicamentos eficazes para cicatrização. Este artigo demonstra a obtenção de emulgel fitoterapêutico composto por óleo de copaíba (*Copaifera martii*) e barbatimão (*Stryphnodendron adstringens* (Mart.) Coville) incorporado em uma blenda polimérica composta por Pluronic® F127 e Carbopol® 934P. A formulação mostrou potencial antimicrobiano inativando a bactéria *Staphylococcus aureus.* A administração do fitoterápico no umbigo de cordeiros recém-nascidos durante cinco dias mostrou adequada cicatrização, não permitindo a ocorrência de inflamação ou miíase. O eritrograma e leucograma dos animais mostraram equivalência estatística entre os animais tratados com o fitoterápico e iodo. Ainda, o índice de intervenção humana do emulgel fitoterapêutico foi menor quando comparado ao iodo. O conjunto de resultados evidenciaram o potencial do medicamento desenvolvido, sendo um candidato para outros ensaios na clínica veterinária.

TERMOS DE INDEXAÇÃO: Miíase, óleo de copaíba, barbatimão, feridas, cicatrização de umbigo, ovinos.

INTRODUCTION

According to the "*Instituto Brasileiro de Geografia e Estatística*" (Brazilian Institute of Geography and Statistics – IBGE), the herds

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of cattle, sheep, pigs, goats, and buffaloes increased by up to 13% in 2020 (IBGE 2020). While these numbers position Brazil prominently, there is room for improvement as the Brazilian livestock population is severely affected by disease-related losses, which can amount to billions of dollars. New veterinary medications are being introduced into the market daily, aiming to facilitate universal and efficient management practices, reduce production losses, and bolster the Brazilian livestock economy.

In veterinary clinics, cutaneous lesions, wounds, and ulcers represent a significant economic impact due to the high cost of treatments and decreased productivity. Notably, these lesions serve as entry points for microorganisms, which can lead to localized or disseminated infections. A common aggravating factor in Brazilian herds is the infestation by larvae from the *Cochliomyia hominivorax* fly. This pathological condition is known as myiasis and describes the presence of larvae that infiltrate, feed on, and evolve as parasites (Da Silva et al. 2005, McBride & Walker 2018). Infected wounds caused by larvae rapidly increase in size due to consuming muscle tissue, connective tissue, blood vessels, nerves, and cartilage. These wounds are always accompanied by bacterial infections, which can lead to death if not treated quickly and effectively (Juyena et al. 2013, Yusuf et al. 2019, Milne & Penn-Barwell 2020). Ivermectin is a widely used antiparasitic medication in veterinary clinics. It is an injectable product considered the most commonly used worldwide due to its broad spectrum against internal and external parasites. However, recent studies have indicated neurological risks in humans and animals associated with using ivermectin (Powrie et al. 2022), making it urgent to develop more tolerable and safe treatments for systemic and topical use.

By harnessing the therapeutic potential of medicinal plants, the field of phytotherapy has achieved therapeutic efficacy. In this regard, the prevalent use of copaiba resin oil (CRO) extracted from incisions in the trunk of the copaiba tree is noteworthy (Carvalho et al. 2005, Santos et al. 2012, Valadas et al. 2018). In Brazil, the recognized *Copaifera* species include *C. duckei, C. glycycarpa, C. guyanensis, C. martii, C. multijuga, C. paupera, C. piresii, C. pubiflora*, and *C. reticulata* (Martins-da-Silva et al. 2008). Also known as "balm of the Amazon," CRO is rich in sesquiterpenes (volatile fraction) and diterpenes (resinous fraction), classes of substances that possess anti-inflammatory, wound healing, and antimicrobial properties (Pieri et al. 2009, Carvalho & Milke 2014, Rodrigues et al. 2014, Rodrigues Santana et al. 2014). The recognition of the potential of this natural substance has led to its classification in the "Relação Nacional de Plantas Medicinais de Interesse ao SUS" (National List of Medicinal Plants of Interest to the Unified Health System – RENISUS) as having potential for the production of phytotherapeutic medications.

Furthermore, "barbatimão" extract has gained prominence in phyto-cosmetic formulations regarding bactericidal and wound healing properties. *Stryphnodendron adstringens* (Mart.) Coville, a plant species belonging to the Fabaceae family, is rich in tannins (20-30%), flavonoids, alkaloids, starch, resinous and mucilaginous materials, red dye, and tannic acid, as reported in its composition. Infusions of the thick bark have demonstrated beneficial effects on wound healing by promoting tissue repair by associating condensed tannins with proteins in the injured tissues (Ricardo et al. 2018). Furthermore, tannins also offer vasoconstrictor and anti-inflammatory properties that promote the re-epithelialization process in topical wounds. The presence of secondary metabolites derived from the class of tannins allows for antiseptic and antioxidant effects, while proanthocyanidins inhibit *Candida albicans* (Meira et al. 2016, Pereira Junior et al. 2020).

In light of the above, this article focuses on combining natural substances from the Brazilian fauna (from the regions highlighted on the map – Fig.1) to obtain a semisolid composition that can potentially treat skin lesions (whether aggravated by myiasis or not) and promote umbilical cord healing (Fig.1).

MATERIALS AND METHODS

Ethical approval. All procedures followed the current environmental legislation through the "Sistema de Autorização e Informação em Biodiversidade" (National System of Authorization and Information on Biodiversity - SISBIO no. 72922-1) and the "Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado" (National System for Management of Genetic Heritage - SISGEN no. A0F0D20; A7507D7). The studies conducted at the Iguatemi Experimental Farm using newborn Santa Inês lambs were previously approved by the Committee on Ethics in Animal Use under protocol number 2268101221 at "Universidade Estadual de Maringá" (UEM).

Materials. Pluronic® F127 was acquired from Sigma-Aldrich (St. Louis/MO, USA), Carbopol C934P® from Lubrizol Advanced Materials (São Paulo/SP, Brazil), and triethanolamine from Synth (Diadema/SP, Brazil). "Barbatimão" (*Stryphnodendron adstringens* (Mart.) Coville) in powdered form was purchased from a local supplier. Copaiba oil (*Copaifera martii*) was obtained from "Copaíba da Amazônia" company. Brain heart infusion (BHI) broth was acquired from Himedia (São Paulo, Brazil), and Mueller Hinton (MH) broth and agar were acquired from KASVI® (São José dos Pinhais, Brazil). The Chemitec® gel was purchased from a local agricultural store. The composition described for Chemitec® includes 15g of zinc oxide, 2g of cresylic acid, and a sufficient quantity of excipient to make 100g.

Obtaining the phytogel. The "barbatimão" infusion was initially prepared (97g of "barbatimão" powder and 800mL of water at 70°C). Subsequently, the infusion was filtered, and its density and concentration were determined using gravimetric methods. The formulation was prepared using 76.4% w/w of "barbatimão" infusion, 1.2% w/w of Carbopol C934P, 2.4% w/w of Pluronic® F127 copolymer, and 20% w/w of copaiba oil for gel preparation. The mixture was vigorously stirred for 30 min, and the pH was neutralized with triethanolamine.

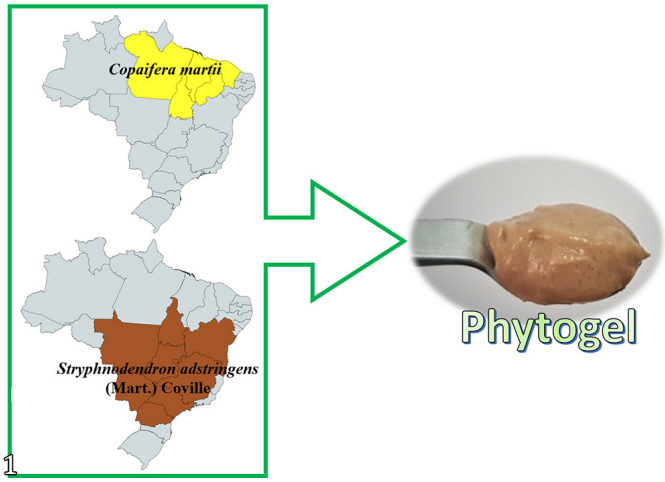

Fig.1. Obtaining phytogel as a veterinary medication.

Texture profile analysis. Texture profile analysis (TPA) was performed to determine the textural parameters of the phytogel, including hardness, cohesiveness, adhesiveness, elasticity, and compressibility. The analysis used a TA-XTplus Texture Analyser (Stable Micro Systems, Surrey, UK). The phytogel, placed in cylindrical glass containers, was compressed twice using an analytical probe (2mm/s compression rate; 15mm depth) with a resting period of 15 seconds between the first and second compressions. Data treatment followed the reported methodology (Ferreira et al. 2017). The evaluations were carried out at room temperature, at 25±2°C. In addition, the commercial veterinary product Chemitec®, designed for topical administration, was analyzed for comparison purposes.

***In vitro* studies: Microbiological analysis of "barbatimão" infusion and phytogel.** The *in vitro* assay used the Gram-positive bacterium *Staphylococcus aureus* (ATCC 25923), previously cultured in BHI broth at 37°C for 24 h. The cell density was standardized for the tests in tubes containing sterile 0.9% saline solution (m/V). The turbidity obtained was equivalent to the reference of the McFarland scale, corresponding to $1x10^8$ CFU/mL (Silva Júnior et al. 2019).

The microbiological analysis was based on the methodology proposed by Campanholi et al. (2021), with some modifications (Campanholi et al. 2020, 2021). In 24-well plates, 1mL of MH broth and 3g of the phytogel were added. First, the mixture was homogenized using a sterile spatula. Then, 1mL of the *S. aureus* suspension was added to each well. Next, subculturing was performed by adding 1mL of the culture to a Petri dish containing MH agar. The plates were incubated at 37°C for 24 h. The same procedure used only 3g of the F127/C934P gel prepared without the natural bioactive. The aqueous "barbatimão" (*Stryphnodendron adstringens* (Mart.) Coville) infusion (5.74mg/mL) was evaluated similarly. However, 1mL of the infusion solution was added to the MH broth well before subculturing. The positive control was performed by adding only the inoculum. After incubation, the total bacterial count was determined in CFU/mL, and the difference between treatments was analyzed using the t-test to evaluate the responses (Campanholi et al. 2022a, 2022b, 2022c).

***In vivo* studies: Navel healing of newborn lambs.** The studies were conducted at the Iguatemi Experimental Farm using newborn Santa Inês lambs. During the treatment, the animals were kept on pasture (Fig.2-3). A total of 20 animals were treated, divided into two groups: phytogel (n=10) and 10% w/v iodine tincture (n=10, control). There was no separation between males and females. The newborn lambs were clinically evaluated, and the treatments were initiated, aiming at umbilical healing and inhibition of infection and myiasis. The administrations were performed once a day in the morning for five days.

During the treatments, the animals were evaluated daily for umbilical conditions (abscesses, purulent discharge, lethargy, nasal discharge, epiphora, diarrhea, and hernia, among others). On the last day of treatment, the animals underwent a blood test to observe their hematological (erythrogram) and leukocytic (leukogram) profiles. Blood samples were collected for analysis, and the hemogram was obtained using Nihon Kohden's automated method. For morphological evaluation of the hematological components, blood smears were stained using the Romanowsky (1891) method (Pereira et al. 2015).

Statistical analysis. The data were analyzed using the open-source software R, version 3.6.0, alongside the R Studio interface, version 1.1.463. A t-test was performed with a significance level of $p \leq 0.05$ to compare the formulated product with the commercial product. The results are presented as mean ± standard error of the mean (Campanholi et al. 2021, 2020).

RESULTS AND DISCUSSION

The extraction of "barbatimão" resulted in a solution with a density of 0.985±0.003g/cm^3 at 25°C and a concentration of 5.74±0.76mg of extract per mL. The herbal formulation obtained from the extract and copaiba oil exhibited a homogeneous and opaque nature due to the formation of microscopic oil droplets dispersed in the cross-linked polymeric matrix of Carbopol (Fig.4-5). The addition of F127 to the matrix was intended to increase the shelf life of the emulsion, as phytogel are thermodynamically unstable and tend to separate phases in a short period.

The phytogel had its textural properties evaluated to predict its behavior regarding physical stability and adhesion to the skin of the animals. In addition, the textural characteristics were compared with those of a commercial veterinary ointment for topical use (Chemitec® brand). The hardness of the gels indicates consistency and firmness, measuring 0.09±0.01 N for the phytogel and 0.28±0.01 N for the Chemitec®. As

Fig.2-3. Sheep kept on pasture.

for the adhesiveness of the phytogel, the results showed a value of 0.45±0.01 N.mm, while the commercial gel exhibited values of 1.46±0.05 N.mm, values close to those reported for dermatological gels (Campanholi et al. 2021, 2022a, 2022b, 2022c). This indicates that the Chemitec® product is more resistant to deformation, a characteristic that also results from its higher viscosity.

Regarding compressibility, the developed formulation showed an average value of 0.49±0.03 N.mm, lower than that of 1.87±0.02 N.mm observed for the commercial product and other formulations reported in the literature. As for the cohesiveness of the emulsion, an average value of 0.87±0.03 (dimensionless) was recorded for phytogel, while the veterinary ointment exhibited a value of 0.81±0.03. These results indicate a more excellent resistance to rupture and stability of the formulated gel than the commercially available product and other studies (Jones et al. 2009, Francisco et al. 2020, Campanholi et al. 2022a, 2022b, 2022c).

Concerning the ability to return to the initial configuration or elasticity, the formulations exhibited similar values of 0.99±0.00mm. At the same time, the commercial product exhibited a value of 1.00±0.00mm, which is comparable to other studies in the literature that used different polymers (Barbosa et al. 2021, Campanholi et al. 2021, 2022a, 2022b, 2022c).

***In vitro* studies: Microbiological analysis of "barbatimão" infusion and herbal gel**

The results of the *in vitro* assays against *S. aureus* (Fig.6) demonstrated bactericidal potential for the "barbatimão" extract present in the herbal gel formulation. The cells remained viable in the positive control, and the F127/C934P polymers were present. The aqueous "barbatimão" solution exhibited a total microbial count of 8.34±0.02 CFU/mL. In contrast, the phytogel containing simultaneous copaiba oil and "barbatimão" resulted in a value of 8.23±0.01 CFU/mL, indicating a statistically significant reduction ($p<0.05$) compared to the positive control (9.83 CFU/mL). The analysis showed that the incorporation of "barbatimão" into the semisolid polymeric matrix and the addition of copaiba resin oil (20% w/w) did not modify the bactericidal potential ($p>0.05$) of the system. However, it is worth noting that while the herbal gel demonstrates bactericidal capacity, the significant advantages of the bioactive contained in the herbal gel lie in their wound healing, reepithelization, and repellent properties, as extensively reported (Carvalho & Milke 2014, Passaretti et al. 2016, Lucas et al. 2017, Debone et al. 2019).

***In vivo* studies: Healing of newborn lambs' umbilical cords**

Regarding the *in vivo* studies, no umbilical pathologies were observed, and the animals exhibited behavior within the normal range for their age group. A 10% w/v iodine tincture solution was selected as the control due to its widespread use in the management of newborns. With the administration of the herbal gel, the gel was found to remain at the treatment site, forming a thick film that acted as a physical protective barrier. On the other hand, the administration of iodine tincture (a liquid widely used in management) resulted in the drainage of the medication in the lambs. In addition, male animals require manual protection of the genital organ during the administration of iodine tincture to prevent contact hypersensitivity. In this aspect, a lower level of human intervention was observed when administering the herbal gel. Approximately 40% of the lambs treated with the herbal gel were completely healed between the second and fifth day of treatment. In comparison, 30% of the animals treated with iodine showed healing within the same period. The photograph was taken on the last day of treatment (Fig.7), using the herbal gel clearly showed a significant contraction of the lesion, which was attributed to the presence of tannins and sesquiterpenes from copaiba oil (Carvalho et al. 2005, Nerio et al. 2010, Meira et al. 2016, Símaro et al. 2020). Neither of the treatments led to the occurrence of myiasis. The average rectal temperature values were 38.7±0.4°C for the animals treated with the natural emulgel and 38.6±0.4°C for the iodine treatment, which is within the species' standard range (Lima et al. 2015).

The blood examination results supported the clinical profile within the normal range of the treated animal groups (Table 1).

From the blood analysis (Table 1), no anemic condition was observed in any treatments, as red blood cells, hemoglobin, and hematocrit levels were within the reference range. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) showed no statistically significant differences ($p>0.05$) between treatments, with both parameters falling within the reference limits for the species. The mean value of total plasma protein (TPP) for animals undergoing the herbal treatment was 6.4±0.8g/dL. In contrast, the iodine treatment resulted in 5.5±0.3g/dL, with the reference range for the species being between 6.0 and 7.5g/dL. The TPP results were similar to those obtained by Pereira et al. (2015) and Lima et al. (2015), which are considered characteristic of the animal's age range. As for platelets, the mean value of the group treated with iodine was 20% higher than the reference range, while the animals subjected to herbal treatment showed a 7% increase in this parameter. However, the statistical comparison between the treatments revealed no significant differences ($p>0.05$). Lima et al. (2015), when studying Santa Inês sheep of different age groups and sexes, obtained platelet values close to 642.10^3/μL for animals aged three to six months raised in a semi-intensive system in the Amazon biome. According to

Fig.4-5. Phytotherapeutic formulation composed of copaiba oil and "barbatimão".

the authors, erythrocyte parameters are influenced by the animal's age range, while gender does not influence these parameters (Lima et al. 2015).

Regarding the neutrophil (N) to lymphocyte (L) ratio, both treatments showed a predominance of neutrophils (N:L ratio >1). This result was expected since neutrophils are predominant in young sheep, while lymphocytes predominate in adults. The overall leukocyte count was similar to that reported in other studies and within the reference values for the species (Madureira et al. 2013, Lima et al. 2015). The values of myelocytes, metamyelocytes, basophils, and band cells were zero in both treatments. Additionally, basophilic stippling was observed in the differential leukocyte count. Hemoparasite screening was performed on blood smears, yielding negative results. Although the quantitative aspects showed statistical equivalence between the treatments (p>0.05), the management of animals subjected to the administration of the herbal gel was facilitated, and the medication met the antimicrobial, repellent, and healing needs required in the treatment of lambs. As future prospects, acute and subchronic dermal toxicity studies of the phytogel will be conducted to determine the biological safety of the product.

Table 1. Blood analysis (mean±standard deviation) collected during the experiment from neonatal lambs

	Phytogel	Iodine
Red cell (/µL)	8.98±0.99	8.50±1.09
Hemoglobina (g/dL)	12.0±1.0	11.6±1.5
Hemoglobin (%)	36.3±3.5	36.0±4.0
MCV (fL)	40.2±0.8	43.0±1.9
MCHC (g/dL)	33.1±0.4	32.3±1.0
Platelets (/µL)	649333±88596	750667±161571
TPP (g/dL)	6.4±0.8	5.5±0.3
Leucocytes (/µL)	4800±608	6266±2732
Myelocytes (%)	0±0	0±0
Metamyelocytes (%)	0±0	0±0
Rods (%)	0±0	0±0
Neutrophils (%)	58.3±11.0	65.1±6.0
Lymphocytes (%)	38.7±11.7	31.7±8.1
Monocytes (%)	2.7±0.6	2.7±3.1
Eosinophils (%)	0.3±0.6	0.6±1.1
Basophils (%)	0±0	0±0

MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, TPP = total plasma protein.

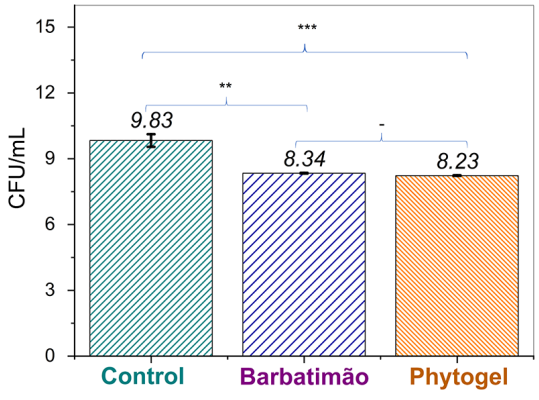

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Fig.6. Total count of *Staphylococcus* aureus expressed in log CFU/mL for control, aqueous extract of "barbatimão", and phytogel, where p<0.01 (***), p<0.04 (**), and p>0.05 (-).

CONCLUSION

The phytogel showed similar adhesiveness and hardness to the Chemitec® commercial veterinary formulation. *In vitro* tests demonstrated the bactericidal potential of the herbal gel, primarily attributed to the use of "barbatimão". *In vivo* studies revealed effective healing of the lamb's umbilical cord, with a shorter umbilical stump drop time. These findings support the benefits of the combination of "barbatimão" and copaiba oil, highlighting the potential of this medication for both human and veterinary clinical practice.

Authors' contributions.- Ranulfo C. Silva Júnior: Conceptualization, formal analysis, writing – original draft. Karmel P. Pelissari: Formal analysis. Flávia Amanda P. Morais: Investigation, formal analysis. Mônica Regina S. Scapim: Resources, supervision. Fernanda G. Valenciano: Investigation. Henrique L. Perez: Supervision, investigation, formal analysis. Rogério Aleson D. Bezerra: Investigation. Magali S.S. Pozza: Resources, supervision, formal analysis. Wilker Caetano: Resources, supervision. Katieli S.S. Campanholi: Supervision, formal analysis, investigation and writing – original draft.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

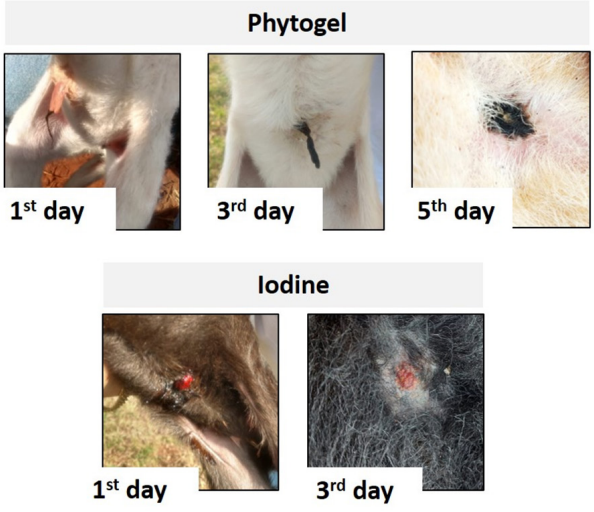

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Fig.7. Representative photo showing the evolution of animals treated with the herbal gel and iodine

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