



Evaluation the effect of use of different levels of mallow (*Malva Sylvestris*) derived extracts on cutaneous skin lesions healing in Simmental heifers

*Avaliação do efeito do uso de diferentes níveis de extratos derivados de malva (*Malva Sylvestris*) na cicatrização de lesões cutâneas em novilhas Simental*

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Abstract: To determine the effect of different levels of mallow derived extracts on Simmental heifer's cutaneous skin lesions by second intention, forty Simmental heifers by average age, 16 months and average weight of 270 ± 45 kg (mean+SD) were selected to evaluate cutaneous lesions healing after treatment with different levels of mallow derived extracts. Experimental wound excision model in Simmental heifers was created by means of a skin punch of 2 cm diameter. The heifers were topically treated for 18 days with a saline control or decoctions of mixed with common thistle and thyme derived extracts with carboxymethyl cellulose. The centripetal retraction, clinical, and histological aspects of the wounds were observed until complete healing. The phototherapeutics agent presents in thyme improved cicatrization of cutaneous lesions in cattle skin during the first days of treatment. The treatments were beneficial to the reparation process. The phytotherapeutics agent present in mallow derived extracts showed positive effects in the inflammatory phase and on the reparation process. The mallow derived extracts at 200 mg showed a positive effect on the macroscopic aspect of cutaneous lesions in heifers only during the first treatment days. In conclusion different levels of mallow derived extracts improved fibroplasia and its pytotherapeutic activity may be useful in topical treatment of cutaneous lesions in Simmental heifers

Keywords: Cutaneous lesions healing, Mallow derived extracts, Simmental heifers.

Resumo: Para determinar o efeito de diferentes níveis de extratos derivados de malva em lesões cutâneas de novilhas Simental por segunda intenção, quarenta novilhas Simental com idade média de 16 meses e peso médio de 270 ± 45 kg (média + DP) foram selecionadas para avaliar a pele cicatrização de lesões após tratamento com diferentes níveis de extratos derivados de malva. Modelo experimental de excisão de feridas em novilhas Simental foi criado por meio de uma punção de pele de 2 cm de diâmetro. As novilhas foram tratadas topicamente por 18

dias com um controle salino ou decocções misturadas com cardo comum e extratos derivados de tomilho com carboximetilcelulose. A retração centrípeta, os aspectos clínicos e histológicos das feridas foram observados até a completa cicatrização. O agente fototerápico presente no tomilho melhorou a cicatrização de lesões cutâneas em pele bovina nos primeiros dias de tratamento. Os tratamentos foram benéficos para o processo de reparação. O fitoterápico presente nos extratos derivados de malva apresentou efeitos positivos na fase inflamatória e no processo de reparação. Os extratos derivados de malva na concentração de 200 mg apresentaram efeito positivo sobre o aspecto macroscópico das lesões cutâneas em novilhas apenas nos primeiros dias de tratamento. Em conclusão, diferentes níveis de extratos derivados de malva melhoraram a fibroplasia e sua atividade fitoterápica pode ser útil no tratamento tópico de lesões cutâneas em novilhas Simental

Palavras-chave: Cicatrização de lesões cutâneas, Extratos derivados de malva, Novilhas Simental.

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Introduction

Cutaneous wound healing occurs in a really complex process (ARMSTRONG & JUDE, 2002). Wound healing processes consist of integrated cellular and biochemical cascades leading to reestablishment of structural and functional integrity of the damaged tissue (BOLOGNIA et al., 2008). It takes place in three overlapping phases such as inflammatory, proliferative and remodeling. Several types of inflammatory cells attend to the injury site and in addition to their phagocytic and anti-microbial activity and play an important role in wound healing process. The proliferative phase involves the creation of a permeability barrier as well as the establishment of an appropriate blood supply and reinforcement of the injured tissue and remodeling. Deposition of matrix materials and their subsequent change over time is the third phase of wound healing. The utilization of medicinal plants and their derivatives in wound healing is more appropriately recognized as herbais remedy than traditional medicine. For centuries, medicinal herbs have been used for the treatment of healing in many countries (CHANGAEE, et al., 2003; BAZZAZ AND HARIRIZADEH, 2003). Plants have immense potential in the wounds management and treatment and their derived

extracts may increase healing and tissue regeneration through multiple connected mechanisms (Adikwu et al., 2008), and synergistic effect on the overall healing procedures (PAWAR & TOPPO, 2012). Researcher studies showed that the wound healing properties of natural products with anti-inflammatory, antioxidant, antibacterial and pro-collagen synthesis actions have been conducted (HENRY & GARNER, 2003). The herbal properties might be due to the bioactive phytochemical constituents of the various chemical families such as alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds and each bioactive agent may have specific function on wound healing properties (SARABAHI, 2012; DORAI, 2012). The Mallow (*Malva Sylvestris*) is part of the large family of Malvaceae plants that include cotton, okra and hibiscus (ZARGARI, 2001). It is a winter or summer annual or biennial, freely branching at the base, with a prostrate growth habit (BILLETER et al., 1991). Mallow extracts leaves and flowers have been to treat inflammatory diseases of mucous membranes, cystitis, and diarrhea (BARROS et al., 2010). The results of several studies have shown that mallow extract contains different compounds, including phenolic derivatives, flavonoids, terpenoids, catalase

enzymes, sulfite oxidase, omega-3 and omega-6 fatty acids, beta carotene, and vitamins C and E, which have anti-inflammatory and antioxidant, antinociceptive, hepatoprotective, wound-healing, anticancer, anti-inflammatory, and potent antioxidant properties (PIRBALOUTI et al., 2010 ; MOUSAVI et al.,2021). Some studies supported the idea of further wound size reduction in the early days when using the mallow extract (UZMA et al., 2020; EBRAHIMI et al., 2022). These reports also suggest that *Malva sylvestris* is a good candidate for wound healing. However, there are few and somehow controversial studies concerning the effect of this drug on wound healing. Detailed ethnobotanical studies reveal that mallow is widely used in Iran to treat infectious, inflammatory and microbial skin diseases and cure wounds. Additionally in (PIRBALOUTI et al., 2010) study, result showed rat wounds treated with *Malva sylvestris* extract had significant smaller sizes than other groups and collagen fibers in the skin of rats in this treatment group were more organized than the other groups. In the last decade, researches has been focused in the area of wound healing in animal and human and its management through plant derived extract, hereby the aim of this study was to evaluate the different levels of mallow

derived extracts on Simmental heifer's cutaneous skin lesions healing.

Materials and methods

The present study was carried out at Lorestan veterinary research institute during 2022 year. Forty beef Simmental heifers at average age of 16 months and average weight of 270 ± 45 kg (mean+SD) were used in the current study. Heifers were confined to individual stalls in a roofed barn and they were fed by basal diet contains corn silage, mineral and vitamin mixture and water and on the free-choice form. They were confined for two weeks to allow adaptation before initiation of the experiment. The mallow plant were cleared of dirt and dried under shade for about 25 days and using a mechanical grinder and powdered. The obtained powder was extracted with 95% ethanol that was used for extraction as many polar and non-polar compounds can be extracted from the ethanol for 4 days, followed by hot percolation for 4 hrs. Then it was filtered and distilled at 80°C . The extract was transferred into the previously weighed empty china dish and evaporated to get an ethanoic extract and kept in anhydrous calcium chloride containing desiccator and then the percentage yield of the extract was calculated. An isotonic solution of Na-Cl

homogenized with six g of carboxymethyl cellulose was used as control therapy.

Decoctions were prepared only once, and they were stored in amber glass bottles under refrigeration 4–8°C. After 24 h food withdrawal and a 12h water withdrawal wounds were surgically created. Thereafter, the heifers were sedated by 0.04 mg per kg of Xylazine HCL and their hair was clipped from an area of approximately 45×45 cm² in the lumbar region. After sedation, lidocaine was applied to the incision areas, and four full-thickness lesions were made by excising the skin to the level of loose subcutaneous tissue on each side in the lumbar region, using a punch of diameter two cm without antiseptics, thus preserving the resident microbiota. The circular wounds were located at ten cm from the spinal column, and were separated from each other by the same distance ten cm. Clinical treatment was initiated 12 hours after the surgical wounds were made, and was administered on a daily basis until complete cicatrization of the lesions. The treatments were completely randomized, so as to avoid performing the same clinical procedure in the craniocaudally direction. The phytotherapeutics plant decoction condensed with carboxymethyl cellulose were directly applied on the wounds daily with a syringe.

The tails of the heifers were tied to their shins with a string throughout the experiment. The lesions on the right side in the lumbar region of each animal were clinically evaluated for local hemorrhage, presence of clots, crusts, granulation tissue, epithelization, and presence of exudate, and were classified as bad (1), regular (2), or good (3) by the same evaluator throughout the study. The macroscopic evaluation was performed on a daily basis until the 18th day after the surgery. For measure wound retraction, each wound area on days 2, 4, 6, 8, 12, 14, 16, and 18 by placing a transparent plastic sheet on the lesion and marking the surrounding perimeter with a projector pen were measured. The lesions on the left side in the lumbar region of 20 randomly chosen heifers were selected for biopsy. Samples collected from the geometrical center of the lesions by using a surgical punch of diameter 10 mm on days 8 and 18 after wound establishment. The material was fixed in 10% formaldehyde for histopathological analysis. The fragments were stained with hematoxylin and Harris eosin, and analyzed by a pathologist who was blinded to the experimental methodology. For fragments obtained on day 8, inflammatory reaction was evaluated on the basis of cellularity and edema formation.

The presence of young granulation tissue was also evaluated. For cellularity, the following grades were attributed: present (1), moderately infiltrated (2), or severely infiltrated (3). Edema was classified as absent (1), slight (2), or severe (3). The young granulation tissue was classified as traces (1), moderate (2), or abundant (3), by using a semi-quantitative analysis. Treatment averages were obtained from the inflammatory reaction and granulation tissue deposition evaluation grades. The material obtained on day 18 was evaluated for granulation tissue deposition by examining the presence and quantity of young or mature granulation tissue, and was graded as minimum (1), moderate (2), or abundant (3) deposition. Inflammatory reaction was classified as weak (1), strong (2), or severe (3). As on day 7, treatment averages were obtained from the evaluation grades for

inflammatory response and granulation tissue deposition.

Statistical analysis

Data obtained were analyzed using the GLM procedure of SAS 9.12 version. Comparison means differences between treatments were done by t-test were and ($p \leq 0.05$) was considered as a significant. Qualitative parameters from the microscopic analysis were used for defining the healing quality response (STEEL et al., 1997).

Result

Evaluation grade of heifer's skin wounds on days 2, 4, 6, 12, and 18 are shown in Table 1. Data showed that the wounds that treated with mallow extract exhibited serous, smooth, and slender crusts, and borders with less edema and the wounds exhibited significantly better macroscopic characteristics than the control at the beginning and end of the treatment ($p \leq 0.05$).

Table1- Evaluation grade of heifer's skin wounds on days 2, 4, 6, 12, and 18

Days \ Treatments	2	4	6	12	18
Control	1.90 ^{c*} ±0.15	1.96 ^{c*} ±0.15	1.97 ^{c*} ±0.12	1.98 ^{c*} ±0.06	2.05 ^{c*} ±0.07
Mallow 100 mg	1.97 ^{b*} ±0.15	2.11 ^{b*} ±0.14	2.12 ^{b*} ±0.08	2.19 ^{b*} ±0.14	2.24 ^{b*} ±0.10
Mallow 200 mg	2.45 ^{a*} ±0.11	2.38 ^{a*} ±0.17	2.48 ^{a*} ±0.11	2.42 ^{a*} ±0.15	2.62 ^{a*} ±0.14
P- Value	**	**	**	**	**

*a,b,c = Means in the column significantly differed

According to the data presented in Table 2 the wound area averages (cm²) did not significantly differ from average wound area of the control group at any time (p≤0.05). However, the lesion area was slightly smaller in the 200 gr mallow treated

group. The possible reason for enhanced wound healing effect may be due to the 100 and 200 gr mallow derived extracts which may possess antioxidant, free radical scavenging properties and promote cell proliferating properties.

Table 2- Heifer skin wound areas on 2, 4, 12, and 18 days (cm²)

Days \ Treatments	2	4	12	18
Control	3.26±0.05	2.69±0.12	2.24±0.14	1.27±0.19
Mallow 100 mg	3.21±0.18	2.45±0.14	2.11±0.16	0.99±0.14
Mallow 200 mg	3.18±0.21	2.58±0.19	2.12±0.18	1.04±0.12
P- Value	n.s*	n.s	n.s	n.s

*n.s = Means in the column do not significantly differ

Interestingly, the day-8 histological evaluation did not reveal significant differences in inflammatory response and granulation tissue deposition between the control and mallow treatments. The best macroscopic evaluation was not associated with quantifiable alterations in microscopic

evaluation. Microscopic analysis on day 18 revealed that mallow extract at 200 mg was more beneficial to the healing process. Mallow treatment at 200 mg resulted in a greater amount of mature conjunctive tissue than the other treatments (Table 3).

Table3- Histological evaluation numbering of heifers skin wounds on days 8 and 18

Histological Evaluation \ Treatments	Inflammation response 8 days	Inflammation response 18 days	Granulation response 8 days	Granulation response 18 days
Control	1.4±0.2	2.1±0.21	1.9±0.3	1.6±0.4
Mallow 100 mg	1.6±0.5	1.8±0.4	1.6±0.8	2.2±0.4
Mallow 200 mg	1.8±0.3	2.0±0.5	1.7±0.7	2.1±0.8
P- Value	n.s*	n.s	n.s	n.s

*n.s = Means in the column do not significantly differ

Discussion

In present study, results of wound area measurements, indicated a healing potential for the mallow extract.

The area measurements showed that there is significance differences between the different groups. Afshar et al (2015) showed that on days 4 and 7, the numbers of inflammatory cells in the *Malva sylvestris*-treated groups were significantly lower than the control group at the edges of the wound in both groups was significantly higher than the control group. They also showed that on the tenth day of the study, better healing features and less fibrosis and scar formation, and also fewer hair follicles were damaged were observed. In the (Pirbalouti et al., 2010) study result showed that the extract-treated animals by *Malva sylvestris* showed significant reduction in the wound area when compared with other groups. Also, histopathological studies of the tissue obtained on days 6th, 9th and 16th from the extract-treated by *Malva sylvestris* showed increased well-organized bands of collagen, more fibroblasts and few inflammatory cells.

Result of (Houshang najafi et al., 2017) showed the best statistical, clinical, and microscopic healing results with lower inflammatory cells, and more fibroblasts and collagen density in *Malva sylvestris*

treatment compared to other groups. Many studies have shown that *Malva sylvestris* application speeds up wound healing and repair (Afshar et al., 2015; Pirbalouti et al., 2010; Mousavi et al., 2021) and one such study showed that an ordered epidermis covered the total thickness of the wound region with mature scar tissue in the dermis. Farahpour and Sedaghat, (2015) suggested that the significant increase in wound contraction rate was seen in the treatment groups, especially the 3-percentage group, compared to the control group. Moreover, the inflammatory cell score showed a decrease, whereas new vessel formation, fibroblast distribution, collagen production and epidermis thickness increased in *Malva sylvestris* hydroethanolic leaf extract-treated animals. Uzma et al (2020) showed that the results clearly demonstrated that both 5% and 10% *Malva sylvestris* cream was superior to sulfadiazine in terms of reducing the time required for complete wound healing.

Conclusion

Overall, we could demonstrate that the phytotherapeutic agent in mallow may improve cicatrization of cutaneous lesions in experimental heifer's skin during the first days of treatment. The treatments by mallow at 100 and 200 mg were beneficial act to the reparation process. Mallow derived extracts

exhibited positive effects in the inflammatory phase and on the reparation process in wound healing. Also it has a positive effect on the macroscopic aspect of cutaneous lesions in experimental heifers during the first treatment days and mallow extracts improved fibroplasia. Phytotherapeutic activity of mallow at 200 mg was the most superior, and mallow at the present dosage may be used in topical treatment of cutaneous lesions in Simmental heifers.

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