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Evaluation the effect of common thistle (*Cirsium vulgare*) and thyme (*Thymus vulgaris*) derived extracts on cutaneous lesions healing in Holstein heifers

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ABSTRACT

Background & Aim: Skin wounds are frequently encountered in heifers because of management practices that use bloody interventions, or owing to lesions caused by unsound installations or fights for establishment of herd hierarchy and lead to the development of myiasis with potential economic losses. Plants derived extracts increase healing and tissue regeneration through multiple connected mechanisms and have synergistic effect on the overall wound healing efficiency. Thyme and common thistle active compounds derived extracts have significant effects for proper wound remedy. The aim of this study was to determine the effect of common thistle and thyme derived extracts on Holstein heifer's cutaneous skin lesions by second intention.

Experimental: The twenty Holstein heifers by average age, 15 months and average weight of 250±50 kg were selected to evaluate cutaneous lesions healing after treatment with common thistle and thyme derived extracts. Experimental wound excision model in heifers was created by means of a skin punch of 2cm diameter. The heifers were topically treated for 17 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.

Results: 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 thyme and common thistle derived extracts exhibited positive effects in the inflammatory phase and on the reparation process. The herbal extract exhibited a positive effect on the macroscopic aspect of cutaneous lesions in cattle only during the first treatment days.

Recommended applications/industries: Both thyme and common thistle extracts improved fibroplasia. Phytotherapeutic activity of thyme was the most superior, and they may be used in topical treatment of cutaneous lesions in Holstein heifers.

1. Introduction

Skin wounds are encountered in heifers as a result of management practices that use bloody interventions, or owing to lesions caused by unsound installations or fights for establishment of herd hierarchy (Bowler, 2002). These lesions are often neglected, and lead to the development of myiasis (McAnulty, 2007). The wound healing is an important, multifaceted and physiological process governed by sequential and

overlapping phases, which comprises a series of complex mechanisms activated after the injury. Wound healing is an evolutionary, complex and multicellular process (Guo and Dipietro, 2011). The healing process in five phases such as coagulation, inflammation, proliferation, wound contraction and remodeling (Pazyar *et al.*, 2014). For many years, plants and their various preparations have been used traditionally in relation to wound treatment, especially due to their immense potential to affect the wound healing process (Matthew, 2014; Sarabahi, 2012).

Plant derived extracts may increase healing and tissue regeneration through multiple connected mechanisms, and synergistic effect on the overall healing procedures (Pawar and Toppo, 2012).

Thyme (*Thymus vulgaris* L.) is used in folk medicine, phytopharmaceutical preparations, and also used for its antihelminthic, expectorant, antiseptic, antispasmodic, antimicrobial, antifungal, antioxidative, antivirotic, and carminative effects (Soltani, 2004). It was also used in concoctions that were applied directly to plague blistered skin.

Thymol is a naturally occurring phenol monoterpene derivative of cymene and isomer of carvacrol. It is one of the major constituents of essential oils of thyme, a medicinal plant with several therapeutic properties (Zargari, 2001). Braga *et al*. (2006) mentioned that thymol has significant effects in controlling the inflammatory mechanism present in many infections, which are essential for proper wound remedy. The antiseptic compounds caryophyllene, camphene and thymol found in thyme will guard wounds against infections and speed up wound healing (Alam *et al.*, 2011). Busti *et al*. (2005) showed that the antimicrobial action of thyme is normally resulted by disturbing the function of the cytoplasmic membrane, disrupting the active transport of nutrients to the cell membrane, and coagulation of microbial cell contents. The common thistle (*Cirsium vulgare*) is also known as the bull thistle or spear thistle (Zargari, 2001). It has been used for a variety of medicinal purposes traditionally (Dorai, 2012). The different types of thistles are used to prepare decoctions to alleviate inflammation and an infusion prepared with the leaves and roots of common thistle is believed to heal stiff neck, seizures as well as nervous disorders (Bazzaz and Haririzadeh, 2003). The roots have been employed as a poultice and a decoction prepared using the plant too is used as a poultice to treat aching jaws. It has been traditionally used to treat rheumatic joint pains and a decoction prepared with the whole plant has been used internally as well as externally to heal bleeding piles. The active constituents of common thistle are silybin, silydianin, and silychristin flavonoids. The demulcent found in common thistle has an antioxidant and anti-inflammatory activity (Silva and Fernandes, 2010). Samanta *et al*. (2016) study results showed that the application of silibinin hydrogel for 8 days led to 56.3 percentage wound contraction compared to 64.6 percentage using standard mega heal gel with a subsequent increase in hydroxyproline content, which was significantly higher over control animals. Toklu *et al.* (2007) performed a study on silymarin of milk thistle effect on oxidative damage caused by burns in rats and showed that silymarin can be considered as an adjunctive therapy or replacement in the burn wounds.

It should be noted that the wound healing process can be facilitated by natural products with medicinal properties (Henry and Garner, 2003). Many studies on the wound healing properties of natural products with anti-inflammatory, antioxidant, antibacterial and pro-collagen synthesis actions have been conducted (Kondo *et al.*, 2010). Herbal plant medicinal 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 (Dorai, 2012). In recent years, extensive research has been carried out in the area of wound healing and management through plant derived medicinal products. During the last decades, many attempts have been made to obtain effective new compounds especially herbal extracts, for treatment of wounds that would be economical, applicable topically to the lesions, and could avoid resistance development, here the aim of this study was to evaluate the effect of bull thistle and thyme derived extracts on Holstein heifer's cutaneous skin lesions by second intention.

2. Materials and Methods

2.1. Study setting

The present study was carried out at the field of agricultural organization, Isfahan veterinary research institute during 2017 year. The current study was done by twenty beef cattle heifers from the Holstein breed,

average age of 15 months and average weight of 250±50 kg (mean+SD).

2.2. Experimental animals

The experimental heifers were confined to individual stalls in a roofed barn located in Isfahan, Iran. The heifers were fed by basal diet contains corn silage, mineral and vitamin mixture and water and a concentrated containing 18% crude protein and 75% total digestive nutrients on the free-choice form. In addition, the animals were confined for two weeks to allow adaptation before initiation of the experiment.

2.3. Preparation of herbal product

The fresh common thistle and thyme were harvested in spring from Chaharmahal and Bakhtiari province, Farsan county, Iran, and dried on forced ventilation stove at $37^{°C}$ for 96 hours. For the extractions, a fiber digester with a condenser in a decoction closed system was used. The herbal solutions were obtained by boiling 100g of each material in 1L of water for 30 minutes. The forming vapor condenses and was reutilized in the boiling process. The decoction was then filtered and homogenized with 6g of carboxymethyl cellulose. An isotonic solution of NaCl homogenized with 6g 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.

2.4. Wounds

The wounds were surgically created after the animals were subjected to a 24 h food withdrawal and a 12h water withdrawal. Thereafter, the heifers were sedated by 0.04 mg per kg of Xylazine HCL and hair was clipped from an area of approximately 60× 60 cm in the lumbar region. After sedation, lidocaine was applied to the incision areas, and 4 full-thickness lesions were made by excising the skin to the level of loose subcutaneous tissue on each side in the lumbar region, after sedation, lidocaine was applied to the incision areas, and 4 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 2 cm without antiseptics, thus preserving the resident microbiota. The circular wounds were located at 10 cm from the spinal column, and were separated from each other by the same distance 10 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.

2.5. Treatments

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 animals 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. Macroscopic evaluation was performed on a daily basis until the 17^{th} day after the surgery.

2.6. Wound retraction measuring

To measure wound retraction, each wound area was measured on days 3, 5, 7, 9, 11, 13, 15, and 17 by placing a transparent plastic sheet on the lesion and marking the surrounding perimeter with a projector pen. The lesions on the left side in the lumbar region of 10 randomly chosen animals were selected for biopsy. Samples were obtained from the geometrical center of the lesions by using a surgical punch of diameter 7 mm on days 7 and 17 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 7, 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 17 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). On day 7, treatment averages were obtained from the evaluation grades for inflammatory response and granulation tissue deposition.

2.7. Statistically analysis

Statistical analysis was performed using the General Linear Models procedure from SAS 9.1 version. For evaluation the differences between treatments the t-test were used and ($p \leq 0.05$) was considered as a significant. Additionally, qualitative parameters from the microscopic analysis were used for defining the healing quality response.

3. Results and discussion

Data from Table 1 showed that the wounds treated with thyme extract exhibited serous, smooth, and slender crusts, and borders with less edema compared to wounds that were treated by the common thistle extract and the wounds exhibited significantly better macroscopic characteristics than the control at the beginning and end of the treatment ($P \leq 0.05$).

Table 1. Macroscopic evaluations of heifers skin wounds on days 3, 5, 7, 13, and 17.

Treatments	Evaluation grade				
	3 days	5 days	7 days	13 days	17 days
Control	1.92^{c*}	1.93^{c}	1.95^{c}	1.99^{c}	2.08^{c}
Thyme	2.39^{a}	2.41^{a}	2.45^{a}	2.48^{a}	2.56^{a}
Common thistle	1.99^{b}	2.05^{b}	2.11^{b}	2.18^{b}	2.21^{b}

*a,b,c : Means in the column are significantly different ($P<0.05$)**.**

The wound area averages (cm^2) did not significantly differ ($P>0.05$) from average wound area of the control group at any time ($P \leq 0.05$)(Table 2). However, the lesion area was slightly smaller in the thyme treated group. The possible reason for enhanced wound healing effect could be due to the crude extracts of thyme and common thistle which may possess antioxidant, free radical scavenging properties and promote cell proliferating properties. The role of antioxidant and free radical scavenging property in wound healing process is further strengthened by other studies conducted on these herbals, which revealed that the plant possesses anti-inflammatory, antipyretic, and antioxidant properties (Gupta *et al.*, 2002). For instance, tannins are seen to be active detoxifying agents and inhibit bacterial growth (Jurjus *et al.*, 2007). *Samanta et al.* (2016) showed that the silibinin hydrogel showed potent wound healing activity in incision and excision wound models in mice. The terpenoids promote the wound healing process mainly due to their astringent and antimicrobial property and flavonoids are potent antioxidants, free radical scavengers additionally polyphenols and flavonoids also possess anti-inflammatory properties and have antimicrobial activities (Kurahashi and Fujii, 2015).

Table 2. Heifer skin wound areas on 0, 5, 13, and 17 days.

Treatments	Wound area (cm^2)			
	0 days	5 days	13 days	17 days
Control	3.26	2.68	2.22	1.25
Thyme	3.26	2.42	2.10	0.98
Common thistle	3.26	2.51	2.14	1.02
P- Value	n.s	n.s	n.s	n.s

*n.s = Means in the column are not significantly different ($P>0.05$).

Interestingly, the day-7 histological evaluation did not reveal significant differences in inflammatory response and granulation tissue deposition between the control and the other treatments (Table 3). The best macroscopic evaluation was not associated with quantifiable alterations in microscopic evaluation. Microscopic analysis on day 16 revealed that thyme extract was beneficial to the healing process. Thyme treatment resulted in a greater amount of mature conjunctive tissue than the other treatments did. Development of dermal annexes, which may be related to low oxygen tension induced by the tannins, was observed. Fibrin accumulation was also observed in the center of the wound (Armstrong and Jude, 2002). It stimulates the multiplication and centripetal migration of fibroblasts through the fibrin net, suggesting that thyme promoted higher fibroplasty. Micro histological evaluations revealed a positive effect common thistle and thyme on granulation tissue deposition on day 16. Although an improvement in inflammatory control was expected in the herbal group, but also there were not detected in the tissue by microscopy. Data revealed the significant differences in the macroscopic aspects of the wounds in the comparative study of phytotherapeutics, but the average wounds areas did

not significantly differ. The thyme treatment exhibited more efficient healing, suggesting that this is attributed to antibacterial and anti-inflammatory activities of the plant. The results of current study are in line with Braga *et al*. (2006) study that reported thymol as main component of thyme oil has bactericidal effect and can be helpful in regulating the inflammatory mechanism which is required for appropriate wound healing. Since inflammation causes many difficulties including infection, wound dehiscence and impaired collagen synthesis, anti-inflammatory effects of thymol would be promising material when thyme essential oil is used (Barakat *et al.*, 2018). Costa *et al*. (2019) noted that the thyme and common thistle derivatives are able to act in the three phases of wound healing. In the first phase, they showed modulatory effect of the inflammatory cytokines, oxidative stress and antimicrobial power. In the second phase, they promoted re-epithelialization, angiogenesis and development of granulation tissue. Finally, in the third phase, they improve the collagen deposition and modulated the growth of fibroblasts and keratinocytes. Mollarafie *et al.* (2015) showed that incorporating thymol into the dressing increases its elasticity and porosity, but reduces its mechanical strength.

Table 3. Histological evaluation numbering of heifers skin wounds on days 7 and 17.

Treatments	Inflammation response		Granulation response	
	7 days	17 days	7 days	17 days
Control	1.4	2.1	1.9	1.6
Thyme	1.6	1.8	1.6	2.2
Common thistle	1.8	2.0	1.7	2.1
P- Value	n.s	n.s	n.s	n.s

*n.s= Means in the column are not significantly different (P>0.05).

4. Conclusion

In conclusion, we could demonstrate that the phytotherapeutic agent present in thyme can improve cicatrization of cutaneous lesions in cattle skin during the first days of treatment. The treatments were beneficial to the reparation process. The phytotherapeutic agent present in thyme and common thistle derived extracts exhibited positive effects in the inflammatory phase and on the reparation process. The herbal extract exhibited a positive effect on the macroscopic aspect of cutaneous lesions in cattle only during the first treatment days. Both thyme and common thistle extracts improved fibroplasia. Phytotherapeutic activity of thyme was the most superior, and they may be used in topical treatment of cutaneous lesions in Holstein heifers.

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