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Original article

Clinical and immunological effects of *Malva sylvestris* and levamisole combination in the treatment of trichophytosis in cattle

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Abstract

This study investigated the clinical and immunological efficacy of combining *Malva sylvestris* L. extract with levamisole in calves naturally affected by bovine trichophytosis. Forty clinically diagnosed calves (8–11 months old) were randomly allocated into four groups: Control, Malva, Levamisole, and Combination. All animals received subcutaneous ivermectin (0.2 mg/kg) ten days before treatment and were maintained under uniform housing, feeding, and management conditions throughout the study. Treatments were applied for 21 days: Control (distilled water + saline), Malva (*M. sylvestris* extract + saline), Levamisole (distilled water + levamisole, 2.5 mg/kg), and Combination (both treatments). Lesion diameters were recorded on days 0 and 21. Blood samples collected on days 0, 7, 14, and 21 were analysed for leukocyte profiles and serum IgG, IL-6, and IFN-γ concentrations. The Combination group exhibited the most pronounced reduction in lesion size ($p \leq 0.05$) and marked elevations in leukocyte counts and IgG levels ($p \leq 0.01$). IL-6 concentrations significantly decreased in the Malva group by day 21 ($p \leq 0.001$), whereas IFN-γ levels showed notable increases in the Levamisole and Combination groups ($p \leq 0.001$). Overall, these findings underscore the therapeutic potential of integrating topical *M. sylvestris* with systemic levamisole as an effective complementary strategy for managing bovine dermatophytosis.

Keywords: cattle, dermatophytosis, levamisole, malva sylvestris, trichophyton verrucosum

Introduction

Dermatophytosis, commonly known as ringworm, is a superficial fungal infection in cattle primarily caused by *Trichophyton verrucosum*, posing significant zoonotic risks in livestock-production environments (Hizli 2020, Tartor et al. 2020, Abdullah et al. 2021). Transmission typically occurs through direct contact or exposure to contaminated fomites, thereby placing individuals in close proximity to livestock – such as farmers and veterinarians – at elevated risk of infection (Al-Farha and Mahmood 2021).

The disease primarily affects keratinised tissues such as hair, skin, hooves, and horns (Yildirim 2020). Disease progression is influenced by host immune status, genetic predisposition, nutritional factors, environmental conditions, and the administration of immunosuppressive agents (Tartor et al. 2020, Abdullah et al. 2021). Clinical manifestations include alopecia, exudative plaques, and thickened greyish-white lesions, typically localised to the head and neck, though they may extend to other anatomical regions (Yildirim 2020, Al-Farha and Mahmood 2021). The disease also imposes a considerable economic burden on livestock production, arising from decreased productivity and the associated costs of treatment and control strategies (Tartor et al. 2020).

In this context, *Malva sylvestris* L. (Malvaceae) – a phytochemically rich medicinal plant native to Eurasia and North Africa – has attracted considerable attention due to its well-documented anti-inflammatory, antimicrobial, and wound-healing properties (Barros et al. 2010, Feizi et al. 2018, Batiha et al. 2023). Its leaves and flowers are rich in bioactive constituents, including mucilage, flavonoids, tannins, polyphenols, and antioxidants (Hajyani et al. 2015, Jabri et al. 2017), which collectively support its traditional use in the treatment of cutaneous and mucosal disorders.

Levamisole, a well-established anthelmintic agent, is also utilised for its immunostimulatory properties. At immunomodulatory doses (2-2.5 mg/kg), it activates T cells and enhances cytokine-mediated responses, often in combination with vaccination protocols (Siwicki and Cossarini-Dunier 1990, Sajid et al. 2006).

This study hypothesised that the integration of topical *M. sylvestris* extract with systemic levamisole would provide synergistic benefits in the treatment of bovine trichophytosis. Specifically, this combination was anticipated to enhance lesion resolution, increase IgG and IFN-γ responses, attenuate IL-6 – mediated inflammation, and improve haematological indices beyond the therapeutic effects observed with either agent alone.

Materials and Methods

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments at Kafkas University (Approval No: KAÜ-HADYEK/2025-122; 3 June 2025), and with authorisation from the Ardahan Provincial Directorate of Agriculture and Forestry (Document No: E-29486769-325.99-19362885; 22 May 2025).

Preparation of *Malva sylvestris* extract

Aerial parts of *Malva sylvestris* were collected from Ardahan Province (40.8486°N, 42.7192°E; elevation: 2,034 m) in June 2025 and morphologically identified based on external botanical characteristics, including leaf shape and flower morphology, in accordance with previously reported methods (Rhimi et al. 2025). Following shade-drying, 20 g of the plant material was infused in 100 mL of boiling distilled water for 10 minutes, filtered using Whatman No.1 paper, and stored at 4°C (Feizi et al. 2018).

Microbiological analysis

Samples (skin scrapings and hairs) were aseptically collected from the margins of lesions and examined microscopically using 10% KOH, as well as by culture on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) at 37°C for 2-4 weeks. Identification of *Trichophyton verrucosum* was confirmed microscopically via lactophenol cotton blue staining, in accordance with standard mycological procedures (Rudramurthy and Shaw 2017).

Experimental design

Forty calves (aged 8-11 months; mixed sex and breed) suspected of having trichophytosis were clinically examined, and the diagnosis was confirmed through direct microscopic examination and fungal culture of lesion scrapings. Although the ethical approval covered the 3-12-month age range, only clinically affected calves aged 8-11 months were included in the final analysis to minimise age-related variation in immune function. The animals were randomly assigned to four equal groups (n=10): Control, Malva, Levamisole, and Combination. All calves received subcutaneous ivermectin (0.2 mg/kg; Vilmectin®, Vilsan) ten days prior to treatment. All affected animals originated from a single herd and were housed under uniform, standardised environmental conditions with ad libitum access to water and forage. Furthermore, all calves were maintained under identical feeding and management protocols throughout the study period.

Control: Topical application of distilled water (twice daily for 21 days) combined with subcutaneous (SC) administration of 0.9% saline (2 mL; three doses at 3-day intervals).

Malva: *M. sylvestris* extract applied topically (twice daily for 21 days) plus SC saline as in the Control group.

Levamisole: Topical distilled water (as above) combined with levamisole (2.5 mg/kg SC; Levatek 10%, Teknovet®; three doses at 3-day intervals).

Combination: *M. sylvestris* (topical) and levamisole (SC), administered as per the respective monotherapies.

In all treatment groups, topical applications were delivered using a handheld atomizer, sprayed from a distance of approximately 10-15 cm to ensure consistent distribution and complete wetting of the lesion surface.

Lesion areas were delineated using acetate sheets and measured on graph paper on days 0 and 21 (Hizli 2020). To minimize measurement variability related to lesion morphology, all assessments were performed by the same investigator using the maximum transverse diameter of each lesion.

Laboratory analysis

Blood samples were collected via jugular venepuncture on days 0, 7, 14, and 21 into two types of tubes: EDTA tubes (2 mL; BD Vacutainer® K2E 5.4 mg, Franklin Lakes, NJ, USA) and plain tubes without anticoagulant (5 mL; BD Vacutainer® CAT). All samples were transported under controlled cold-chain conditions. Serum was separated by centrifugation (3,000 rpm for 20 minutes) and stored at -20°C.

Total and differential leukocyte counts were conducted using a Thoma counting chamber and Wright-stained blood smears, in accordance with standard veterinary haematological procedures (Voigt and Swist 2011). Serum concentrations of IgG, IL-6, and IFN-γ were quantified using ELISA kits (Bioassay Technology Laboratory, Zhejiang, China), and absorbance was measured at 450 nm using a microplate reader (BioTek ELx800, USA).

Statistical analysis

Data were analysed using IBM SPSS Statistics v20.0 (Chicago, IL, USA). The assumption of normality was assessed using the Shapiro–Wilk test. One-way ANOVA was applied, followed by either Tukey's HSD or Games–Howell post hoc tests, as appropriate. Results are presented as mean ± standard error (SE), with a p-value of ≤ 0.05 considered statistically significant.

Results

At baseline (day 0), no significant intergroup differences were observed in neutrophil, eosinophil, monocyte, lymphocyte percentages, or total leukocyte counts ($p>0.05$), thereby confirming the initial homogeneity of the experimental groups (Table 1). Following treatment (days 7, 14, and 21), marked haematological alterations emerged, particularly in the Malva + Levamisole group, which exhibited notable increases in neutrophil, lymphocyte, and total white blood cell (WBC) counts.

On days 14 and 21, WBC counts in the combination group reached 90.10 ± 1.41 and $92.80 \pm 1.22 \times 10^2/mm^3$, respectively – values significantly exceeding those recorded in the Control and Malva groups ($p\leq0.05$). Lymphocyte proportions on day 21 were also substantially elevated in both the Levamisole ($62.80 \pm 1.31\%$) and combination ($62.60 \pm 1.70\%$) groups. Similarly, neutrophil levels were markedly higher in the Combination group ($35.30 \pm 1.78\%$) compared to the Control group ($28.70 \pm 1.13\%$) ($p\leq0.05$). A pronounced and consistent rise in monocyte counts was observed in the Levamisole-treated groups from day 7 onwards, whereas eosinophil levels remained stable without significant fluctuation. Collectively, these findings indicate robust immunostimulatory activity induced by levamisole, which appeared to be further enhanced by the concurrent topical application of *Malva sylvestris.*

At baseline, IL-6 concentrations were comparable across all groups ($p=0.284$). However, by day 7, a marked and statistically significant decline was observed in the Malva group (65.60 ± 2.74 pg/mL), and this reduction continued progressively to 61.00 ± 1.53 pg/mL by day 21 – values substantially lower than those recorded in the Levamisole (87.10 ± 2.30 pg/mL) and Control groups ($p\leq0.001$). The Malva + Levamisole group displayed an intermediate and partially attenuated IL-6 response (69.70 ± 1.83 pg/mL), consistent with a potential additive anti-inflammatory response (Table 2).

Conversely, IFN-γ levels – initially uniform across all groups ($p=0.461$) – rose sharply in both the Levamisole and Combination groups by day 7 and continued to increase, reaching peak values of 155.60 ± 1.93 and 158.00 ± 1.97 pg/mL, respectively, by day 21 ($p\leq0.001$ vs. Control). The Malva group also demonstrated a modest but statistically significant elevation (131.00 ± 2.03 pg/mL). Collectively, these cytokine trends suggest a dual immunomodulatory mechanism, wherein Malva sylvestris contributes predominantly to IL-6 suppression, while levamisole drives IFN-γ--mediated cellular immune activation.

At baseline, lesion diameters were statistically comparable across all groups ($p>0.05$). By day 21 (Fig. 1), the Control group showed a progressive enlargement

Table 1. Selected hematological parameters in cattle groups.

Days	Parameters	Control	Malva	Levamisole	Malva+Levamisole
Day 0	Neutrophil %	27.20 ± 0.84	28.50 ± 0.80	28.60 ± 1.09	29.80 ± 1.18
	Eosinophil %	2.20 ± 0.51	2.60 ± 0.56	2.90 ± 0.48	2.40 ± 0.54
	Monocytes %	2.10 ± 0.31	2.50 ± 0.50	3.20 ± 0.61	3.40 ± 0.49
	Lymphocyte %	46.40 ± 1.36	48.80 ± 2.56	49.80 ± 1.66	48.50 ± 2.58
	WBC ($x10^2/mm^3$)	77.80 ± 1.76	74.80±1.36	74.80 ± 0.55	75.20 ± 1.08
Day 7	Neutrophil %	28.50 ± 0.77	29.50 ± 1.18	32.30 ± 1.24	32.20 ± 0.91
	Eosinophil %	1.80 ± 0.46	2.40 ± 0.47	2.60 ± 0.49	2.80 ± 0.49
	Monocytes %	1.50 ± 0.26 [a]	2.60 ± 0.47	3.60 ± 0.45 [b]	3.50 ± 0.50 [b]
	Lymphocyte %	48.30 ± 1.81	50.70 ± 2.34	53.90 ± 1.23	54.00 ± 0.81
	WBC ($x10^2/mm^3$)	75.20 ± 1.61 [a]	76.00 ±1.61 [a]	83.30 ± 1.39 [b]	84.10 ± 1.87 [b]
Day 14	Neutrophil %	27.40 ± 1.24 [a]	29.40 ± 1.60	33.30 ± 0.98 [b]	34.20 ± 1.15 [b]
	Eosinophil %	1.60 ± 0.40	2.70 ± 0.47	2.90 ± 0.52	3.10 ± 0.65
	Monocytes %	2.30 ± 0.36	2.70 ± 0.39	3.50 ± 0.50	3.80 ± 0.41
	Lymphocyte %	48.80 ± 1.61[a]	52.30 ± 2.72	56.80 ± 1.34 [b]	59.50 ± 0.93 [b]
	WBC ($x10^2/mm^3$)	79.90 ± 1.12 [a]	77.30 ± 1.57 [a]	88.30 ± 1.07 [b]	90.10 ± 1.41 [b]
Day 21	Neutrophil %	28.70 ±1.13 [a]	29.80 ± 1.59	34.20 ± 1.28	35.30 ± 1.78 [b]
	Eosinophil %	1.30 ± 0.6	2.60 ± 0.42	3.00 ± 0.57	2.40 ± 0.54
	Monocytes %	2.50 ± 0.37	2.40 ± 0.40	3.00 ± 0.39	3.80 ± 0.49
	Lymphocyte %	44.90 ± 2.82 [a]	52.90 ± 1.53 [a]	62.80 ± 1.31 [b]	62.60 ± 1.70 [b]
	WBC ($x10^2 mm^3$)	73.80 ± 1.30 [a]	75.00 ±1.18 [a]	90.60 ± 1.99 [b]	92.80 ± 1.22 [b]

a,b – Values in the same row with different superscript letters are considered significantly different at $p \leq 0.05$.

Table 2. Time-dependent changes in IL-6 and IFN-γ concentrations among treatment cattle groups.

Parameters	Days	Control	Malva	Levamisole	Malva + Levamisole	P-value
IL-6 (pg/mL)	0	71.40±3.32	68.20±2.52	70.80±1.90	74.90±1.43	0.284
	7	77.70±4.31	65.60±2.74 [a]	78.30±1.72 [b]	75.50±1.39 [b]	0.009
	14	79.70±2.53 [a]	64.80±2.21[b]	79.90±2.77 [a]	72.80±1.34 [a]	0.000
	21	80.70±2.75 [a]	61.00±1.53 [b]	87.10±2.30 [a]	69.70±1.83 [c]	0.000
IFN-γ (pg/ml)	0	128.20±0.69	129.70±1.93	130.80±2.48	132.20±1.62	0.461
	7	125.50±1.57 [a]	128.80±0.62 [a]	138.40±2.09 [b]	141.00±2.39 [b]	0.000
	14	120.60±2.03 [a]	129.70±1.85 [b]	146.30±2.59 [c]	148.60±1.14 [c]	0.000
	21	119.60±0.98 [a]	131.00±2.03 [b]	155.60±1.93 [c]	158.00±1.97 [c]	0.000

IL-6 – Interleukin-6, IFN-γ – Interferon-gamma. Values denoted by different superscript letters (a, b, c) within the same row (same day) indicate statistically significant differences between treatment groups ($p \leq 0.05$)

of lesions (46.70 ± 1.35 mm), whereas the Malva and Levamisole groups exhibited clear reductions to 29.20 ± 1.97 mm and 40.60 ± 1.31 mm, respectively. The Malva + Levamisole group demonstrated the most substantial and clinically meaningful improvement, with lesion size decreasing to 21.20 ± 1.28 mm ($p \leq 0.05$ vs. all groups), highlighting a synergistic enhancement of wound-healing dynamics.

As shown in Fig. 2, baseline IgG concentrations did not differ significantly among the groups ($p > 0.05$). However, by day 14, levels in the Levamisole and Combination groups had increased to 20.90 ± 0.43 and 22.20 ± 0.80 mg/mL, respectively – significantly higher than those observed in the Control (17.80 ± 0.66 mg/mL) and Malva (19.00 ± 0.63 mg/mL) groups ($p \leq 0.05$). This elevation persisted through day 21 (Levamisole: 22.50 ± 0.50 mg/mL; Combination: 23.00 ± 0.57 mg/mL), confirming sustained humoral activation induced by levamisole and further enhanced by the combined treatment.

Photographic evidence (Fig. 3) provides a qualitative depiction of lesion morphology before and after

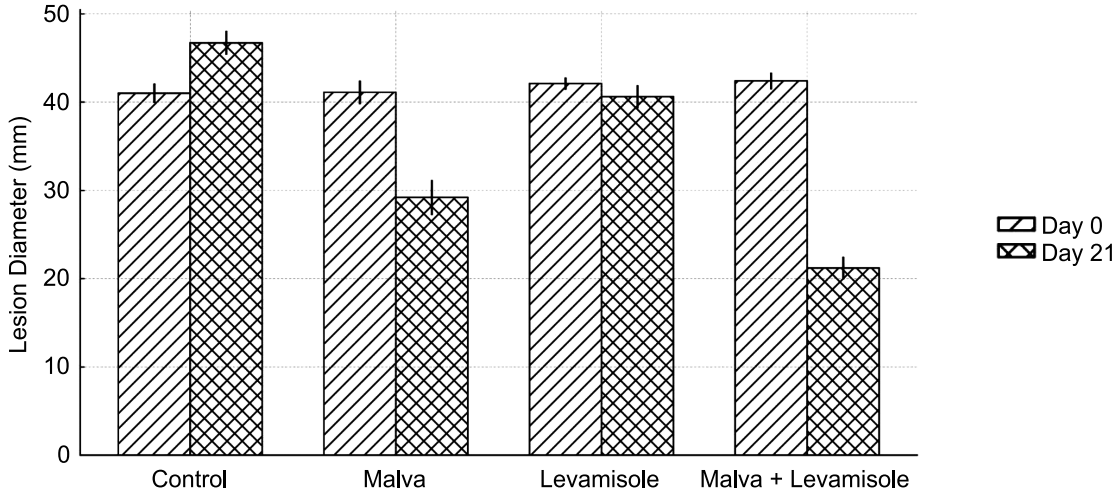

Fig. 1. Lesion diameter (mm) in calves with trichophytosis on day 0 and day 21 across the four treatment cattle groups (Control, Malva, Levamisole, and Malva + Levamisole). Bars represent mean values, and error bars indicate standard error (SE). Day 21 measurements show a significant reduction in lesion size in all treatment groups compared with the Control group, with the greatest reduction observed in the Malva + Levamisole group ($p \leq 0.05$; one-way ANOVA followed by Tukey's HSD/Games–Howell post hoc tests).

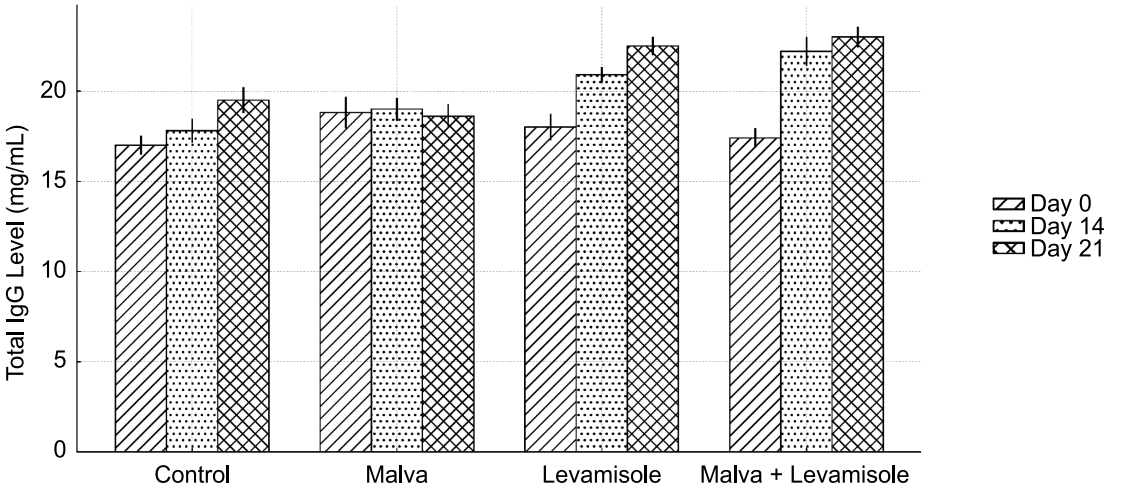

Fig. 2. Serum IgG concentrations (mg/mL) in calves with trichophytosis measured on days 0, 14, and 21 across the four treatment cattle groups (Control, Malva, Levamisole, and Malva + Levamisole). Bars represent mean values, and error bars indicate standard error (SE). IgG levels increased markedly in the Levamisole and Malva + Levamisole groups over the treatment period compared with the Control and Malva groups ($p \leq 0.05$; one-way ANOVA followed by Tukey's HSD post hoc test).

treatment. Minimal visual improvement was observed in the Control group, whereas the Malva + Levamisole group demonstrated marked lesion resolution, characterised by reduced crust formation, diminished lesion borders, and visibly enhanced epithelial regeneration. These visual observations align closely with and reinforce the quantitative lesion measurements, further supporting the clinical superiority of the combined treatment protocol.

Discussion

This study demonstrated that combined treatment with *Malva sylvestris* extract and levamisole significantly enhanced clinical recovery and systemic immune responses in calves affected by trichophytosis. Compared to monotherapies, the combination therapy accelerated lesion healing, elevated serum IgG concentrations, and improved both haematological and cytokine profiles. These findings support the potential utility of integrative immuno-phytotherapeutic strategies in the management of dermatophytic infections. Importantly, the combined protocol also carries substantial clinical relevance, offering a practical therapeutic advantage in cases where conventional antifungal treatments show limited efficacy.

Trichophyton verrucosum, the primary causative agent of bovine dermatophytosis, presents both veterinary and zoonotic risks (Paryuni et al. 2020). Limita-

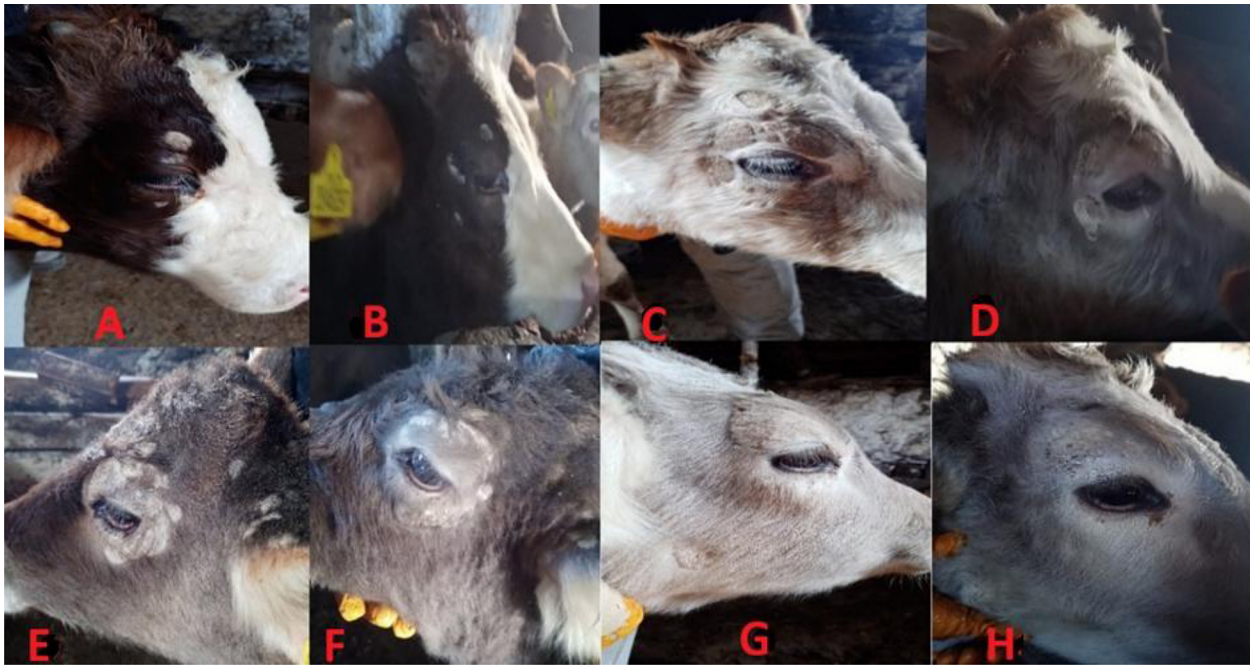

Fig. 3. Representative lesion morphology before and after treatment in the four experimental cattle groups.
(A – Control group pre-treatment, B – Control group post-treatment, C – *Malva* group pre-treatment, D – *Malva* group post-treatment, E – Levamisole group pre-treatment, F – Levamisole group post-treatment, G – *Malva* + Levamisole group pre-treatment, H – *Malva* + Levamisole group post-treatment)

tions associated with conventional antifungal therapies – including prolonged treatment durations, high costs, residue concerns, and emerging resistance – have led to growing interest in alternative strategies involving herbal and immunomodulatory agents (Tartor et al. 2020, Al-Farha and Mahmood 2021).

Malva sylvestris is rich in bioactive constituents – including flavonoids, anthocyanins, tannins, and mucilage – that confer anti-inflammatory, wound-healing, and antimicrobial properties (Barros et al. 2010, Prudente et al. 2017, Batiha et al. 2023). Its therapeutic efficacy has previously been demonstrated in dermatological models (Afshar et al. 2015, Dogan 2023), although its immunological mechanisms remained largely unexplored. In the present study, M. sylvestris not only reduced lesion size but also significantly suppressed serum IL-6 levels, consistent with its established anti-inflammatory profile (Zuo et al. 2017, Wu et al. 2019).

Levamisole, a well-established immunomodulator, enhanced both humoral and cellular immune responses in this study – as evidenced by increased lymphocyte counts, elevated IFN-γ concentrations, and higher IgG levels. These findings are consistent with previous reports of levamisole-induced T cell and monocyte activation, proliferation of bone marrow progenitors, and stimulation of IL-2 production (Sajid et al. 2006, Rao et al. 2017). Notably, the combination therapy demonstrated a synergistic enhancement of IFN-γ, a key cytokine involved in cellular immunity.

Notably, IFN-γ levels were also significantly elevated in the Malva-only group. This may suggest a bidirectional immunomodulatory role of *M. sylvestris*, potentially mediated by antigenic stimulation from fungal cytoplasmic components, as previously reported by Salahi et al. (2020).

Lesion diameter analysis revealed the most pronounced regression in the Combination group (21.20 mm by day 21), corroborating previous findings on topical *M. sylvestris* application (Dogan 2023) and suggesting that systemic support enhances clinical efficacy. Similarly, serum IgG levels peaked in the Malva + Levamisole group (23.00 mg/mL), surpassing those observed in the monotherapy and Control groups. These humoral improvements are consistent with findings from vaccination studies, in which levamisole potentiated IgG production (Sharma et al. 1990, Qureshi et al. 2000, Dogan 2022).

Although oxidative stress markers were not assessed in the present study, the documented antioxidant properties of *Malva sylvestris* suggest that the increase in IgG concentrations observed in the combination group (23.0 ± 0.57 mg/mL) may have indirectly contributed to an enhanced humoral immune response. Colakoglu et al. (2021) reported that oxidative stress levels can influence circulating IgG concentrations, indicating that antioxidant activity may support immunoglobulin synthesis. In this context, the potential antioxidant effects of *M. sylvestris*, together with the known immunomodulatory action of levamisole, may

have contributed to the enhanced IgG response observed in the combined treatment group.

Although the present study was conducted in 8-11-month-old calves, the immunoglobulin kinetics described in early life provide important context for interpreting humoral responses at this developmental stage. Nowak et al. (2012) reported that immunoglobulin levels fluctuate physiologically during the neonatal period as maternal antibodies wane and the foundations of active immunity are established. In this regard, the marked increase in IgG concentrations observed in the Malva + Levamisole group reflects an active immunomodulatory stimulus rather than an age-related or physiological variation, as animals in this age range typically maintain stable immunoglobulin levels. Therefore, the pronounced humoral response detected in the combination group demonstrates that the treatment protocol effectively stimulated active immunity in immunologically maturing calves. These findings further support the possibility that the phytochemical profile of *Malva sylvestris*, together with levamisole's well-characterised immunostimulatory effects, contributed synergistically to the enhancement of immune function observed in this study.

Taken together, these findings indicate that *M. sylvestris* exerts topical effects through anti-inflammatory and antimicrobial mechanisms, while levamisole enhances systemic immunity – culminating in a robust, synergistic response. Given the combination's low toxicity, cost-effectiveness, and ease of administration, it presents a promising and practical alternative for the treatment of dermatophytosis under field conditions.

Recent molecular investigations have shown that PCR-based diagnostic methods substantially improve the detection and species-level identification of dermatophytes, providing a valuable complement to traditional microscopy and culture (Jańczak et al. 2023). In this study, *Trichophyton verrucosum* was identified through both microscopic examination and fungal culture of samples collected from lesion sites. Nonetheless, future investigations may benefit from the inclusion of molecular diagnostic techniques – such as PCR – alongside conventional methods. Such advanced approaches would facilitate a more detailed evaluation of therapeutic efficacy at the microbiological level and further support the integration of phytotherapeutic – immunomodulatory protocols into evidence-based veterinary practice.

A limitation of this study is that lesion depth and crust characteristics were not quantitatively assessed; however, the use of standardised photographic documentation and objective diameter measurements reduced subjectivity.

Conclusion

This study demonstrated that the combination of *Malva sylvestris* extract and levamisole not only accelerated lesion healing but also enhanced systemic immune responses in calves affected by trichophytosis. The synergistic effect was evidenced by reduced IL-6 concentrations, increased IFN-γ expression, and elevated serum IgG levels, lymphocyte proportions, and total leukocyte counts. These findings provide mechanistic and clinical evidence supporting the integration of the anti-inflammatory and antimicrobial properties of *M. sylvestris* with the immunostimulatory actions of levamisole. Collectively, these results provide mechanistic and clinical evidence supporting the complementary roles of topical phytotherapy and systemic immunomodulation in fungal skin infections.

Topical phytotherapy, when combined with systemic immunomodulation, may represent a viable and cost-effective strategy for managing dermatophytic infections in veterinary settings. However, further large-scale, field-based studies incorporating advanced immunological assays are needed to validate and refine this combined therapeutic approach and to determine its broader applicability across different production systems and disease severities.

Author Declarations

Ethics approval

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments at Kafkas University (Approval No: KAÜ-HADYEK/2025-122; 3 June 2025), and with authorisation from the Ardahan Provincial Directorate of Agriculture and Forestry (Document No: E-29486769-325.99-19362885; 22 May 2025).

Use of generative artificial intelligence

In the preparation of this manuscript, the authors utilized ChatGPT (OpenAI) as a generative artificial intelligence (AI) tool. Its use was strictly confined to English language translation, linguistic refinement, grammatical editing, and assistance in the preparation of graphical materials. The AI tool was employed exclusively to enhance clarity, coherence, and overall presentation quality. It was not used for the generation of scientific ideas, data analysis, data interpretation, or the formulation of conclusions. All intellectual input, study design, analytical processes, and final interpretations presented in this manuscript are solely the responsibility of the authors.

Conflict of interest

The author declares that there are no financial, personal, or institutional conflicts of interest that could have influenced the work reported in this manuscript.

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