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Investigation of the Effect of Malva Plant (*Malvasylvestris L.*) on Skin Fungus in Cattle

Ertan DOĞAN[1, *]

[1]*Ardahan University, Vocational School of Göle Nihat Delibalta, Department of Laborant and Veterinary Health, 75700, Ardahan, Türkiye*

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ABSTRACT The purpose of this study is to determine the effect of the *Malva sylvestris L.* (MS) plant on the cutaneous fungus (Trichophytosis, Ringworm) in cattle. The research was carried out on a total of 20 cattle of 6 to 20 months of different races and genders in the Ardahan region. Animals diagnosed with Trichophytosis were divided as control (n=10) and experimental (n=10) groups. No substances were used in the control group. The extract of MS was sprayed with an atomizer onto the lesions of the experimental group. Spraying was done to cover the area where the lesions were located and wet enough. The procedure was conducted twice daily (morning and evening) and for 15 days. Starting from the pre-application (0th day) until the 36th day at intervals of two days, the lesion diameters of the cattle in the control and the experimental group were measured and recorded. At the end of the monitoring period (36th day), the diameter of the lesion was enlarged (29.20±3.58 mm) in the control group and reduced (6.60±5.16 mm) in the experimental group. This difference between the lesion diameters of the control and the experimental groups was statistically significant ($p<0.05$). In this study, it was concluded that the MS extract applied to the bovine skin fungus inhibited the growth of lesions and reduced their size. It is thought that MS extract can be used locally in the treatment of Trichophytosis in cattle.

Keywords:*Cattle, Malva sylvestris, Trichophytosis.*

ÖZ

Malva Bitkisinin (*Malva sylvestris L.*) Sığırlarda Deri Mantarı Üzerine Etkisinin Araştırılması

Bu araştırmanın amacı *Malva sylvestris L.* (MS) bitkisinin sığırlarda deri mantarı (Trikofitoz, Ringworm) üzerine etkisini belirlemektir. Araştırma Ardahan yöresinde ırk ve cinsiyetleri farklı, 6-20 aylık toplam 20 sığır üzerinde yürütüldü. Trikofitoz tanısı konan sığırlar kontrol (n=10) ve deneme (n=10) grubuna ayrıldı. Kontrol grubuna herhangi bir madde uygulanmadı. Deneme grubunda bulunan hayvanların lezyonları üzerine MS ekstresi atomizer ile püskürtüldü. Püskürtme işlemi lezyonların bulunduğu alanı kaplayacak ve yeteri kadar ıslatacak şekilde yapıldı. Bu işleme günde iki kez (sabah ve akşam) ve 15 gün boyunca devam edildi. Uygulama öncesinden (0.gün) başlayıp iki gün arayla 36. güne kadar kontrol ve deneme grubunda bulunan sığırların lezyon çapları ölçülerek kayıt edildi. Takip süresi (36. gün) sonunda kontrol grubunda lezyon çapının büyüdüğü (29.20±3.58 mm), deneme grubunda ise küçüldüğü (6.60±5.16 mm) görüldü. Kontrol ve deneme guruplarında lezyon çapları arasındaki bu fark istatistiksel olarak önemli ($p<0.05$) bulundu. Bu araştırmada sığır deri mantarı üzerine uygulanan MS ekstresi, lezyonların büyümesini durdurarak çaplarını küçülttüğü sonucuna varıldı. Sığırlarda Trikofitoz tedavisinde MS ekstresinin lokal olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler:*Ebegümeci, Sığır, Trikofitoz.*

INTRODUCTION

Fungi cause important diseases which can be observed anywhere in the world. Dermatophytosis (Ringworm) is a fungal disorder that affects the skin surface of cattle (Hizli 2020; Abdullah et al. 2021). It is spread from cattle to humans, affecting human health (Tartor et al. 2020). In particular, animal owners, animal caregivers, technicians, veterinarians and their families are at high risk of contracting the disease. The disease can be transmitted through contact or contaminated tools and utensils (Al-Farha and Mahmood 2021). The disease is primarily caused by fungi such as Epidermophyton, Trichophyton, and Microsporium (Abdullah et al. 2021; Al-Farha and Mahmood 2021). Of these fungal genera, *Trichophyton verrucosum* is the most widespread bovine species and is transmitted to humans (Hizli 2020; Tartor et al. 2020). Skin, nails, hair and horns are most affected by the illness (Apaydin 2020).

✉ *Corresponding author: ertandogan@ardahan.edu.tr

The course and seriousness of the disease depend on the breed, age, parasitic load, care, nutrition and immune status of the animal. Moreover, skin lesions, localization of lesions, environmental factors (humidity, temperature, and crowded environment), certain medications (like cortisol) and the virulence of the fungus determine the prognosis of the disease (Apaydin 2020; Tartor et al. 2020; Abdullah et al. 2021; Al-Farha and Mahmood 2021).

The disease causes substantial economic damage on livestock farms by causing loss of meat, milk, skin and treatment costs (Tartor et al. 2020; Abdullah et al. 2021). It affects cattle of all ages. However, the disease is more common in calves. In diseased cattle, hair loss, exuding on the skin, folliculitis, round, white-grey, thickened lesions occur. Most of these lesions occur in the head and neck. Nevertheless, it may also appear on the back, legs and other parts (Apaydin 2020; Al-Farha and Mahmood 2021).

Malva sylvestris L. (MS), a plant species belonging to the Malvaceae family, grows naturally in the Mediterranean, Asia, northern Africa and European countries. The height of the plant reaches to approximately 100-120 cm, and the length of the flower is up to 2-5 cm (Jabri et al. 2017). The plant is traditionally used in people's food (in the form of soups and salads) and in the treatment of some diseases since antiquity (Barros et al. 2010). Although all parts of the plant are used for medicinal purposes, its leaves and flowers are preferable. It is particularly used in stomach ulcers, expectorant, cough suppressant, laxative, diarrhea-relieving, muscle, skeletal and skin diseases. The plant has anti-inflammatory, anti-oxidant, anti-microbial, anti-septic and anti-cancer properties (Feizi et al. 2018). The most important chemical structure of the plant is made up of mucilage, flavonoids, tannins, tocopherols, ascorbic acid, carotenoids, and anthocyanin (Barros et al. 2010; Hajyani et al. 2015; Jabri et al. 2017).

Silver and silver chloride in the leaves of MS is believed to be effective against *Candida orthopsilos*, *Pasteurella aeruginosa,* and *Bacillus subtilis* (Feizi et al. 2018). It is also suggested that extracts from MS have anti-fungal properties on *Candida albicans* (Cardoso et al. 2012; Alizadeh et al. 2017), *C. Krusei,* and *C. Tropicalis* (Cardoso et al. 2012). In mice infected with *Candida albicans*, the watery extract of MS stimulates the immune system (Hajyani et al. 2015), and is effective against fungi such as *Alternaria alternata*, *Penicillium expansum,* and *Mucor piriformis* (Parveen et al. 2014). This proposed antifungal effect of MS increases the potential for it to be effective against fungi responsible for dermatophytosis in cattle (*Trichophyton verrucosum*, *Epidermophyton spp.*, and *Microsporium spp.*) in vivo conditions. This research was conducted to determine the anti-mycotic effect of MS extract in cattle with dermatophyte (ringworm) diagnosed by clinical and microscopic examination.

MATERIAL AND METHODS

The study has been approved by the Ethics Committee of Kafkas University (decision date 26.01.2023 and numbered KAU-HADYEK/2023-011) and Ministry of Agriculture and Forestry of Türkiye (letter dated 20.12.2022 and numbered E-29486769-325.99-8174016).

MS was collected from the Ardahan region in July 2022. These collected plants were identified by examining their morphological properties (Seçmen et al. 2011). The geographical location of the plant is between 40º 47^{I} and 42^{II} north latitudes, and 42º 36^{I} 32^{II} east longitudes (Google Earth) and its height is 2038 meters. From this area, the aboveground parts of the plant (stem, leaves, flowers, and seed) were collected and laid on a clean newspaper in such a way that they did not overlap. After that, the plants were allowed to dry in the shade and at room temperature. The plants were turned once a day to dry properly. 20 g was taken from the plant, which was found to have dried as a result of physical examination (on the 15^{th} day after the collection day). It was ground into a mortar and placed in Mayer. Then 100 ml of boiling distillate water was added. The cap of the bottle was tightly closed and left in a water bath at 100 °C for 10 minutes. Upon completion of the waiting period, the solution was filtered using Whatman paper (No: 1) (Feizi et al. 2018). The filtrate obtained was placed in a balloon and stored at 4°C in the dark until it was used.

This research was conducted on a total of 20 cattle of different breeds and genders, between 6 and 20 months old, in the Ardahan region. Cattle suffer from dermatophytosis as a result of clinical examination (round, limited, gray-white lesions of different diameters on the skin and hair loss) were divided into control (n=10) and experimental groups (n=10). In the control and experimental groups, care was taken to spread the number of lesions and the diameter of the lesions evenly. Two lesions were found in each of the six animals in the control group, and one lesion was found in each of the four animals. Half of the animals in the experimental group had one lesion, while the other half had two.

Furthermore, two animals in the experimental group had macroscopically small lesions detected. Both groups had lesions in the head, neck, shoulder, and back region. Before beginning the study, body temperature, rumen movement, pulse, and respiratory counts were measured for all animals in the control and experimental groups. These values were found to be within the physiological limits. The control and experimental groups were formed from animals that had never received drug therapy.

The lesioned areas on all animals with suspected trichophytes in both the control and the experimental groups were thoroughly wiped with a cotton soaked in 70% alcohol. After the lesioned area dried, skin scrapings and hair samples were taken from the edges of the lesions with the help of sterile scalpels and pens, placed in a sterile petri dish and the cap was tightly closed. The samples were then brought to the laboratory. The collected samples were placed in the laboratory on 2 drops of 10% potassium hydroxide (KOH) dripped in the middle of a clean slide and the samples were lamellae-covered. The preparation is heated in a slight flame and left for 20-30 minutes at room temperature. The fungi's hyphae and arthrospores were examined under the microscope.

In addition to the microscopic examination, the samples were planted on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) using the artificial stub method. The cultures were kept in an oven at 37 °C for 2-4 weeks and checked every two days for 2-4 weeks. The shape, structure, reproduction rate, and pigmentation status of the colonies were examined in the macroscopic examination of the reproduced cultures. Preparations were made from the cultures and stained with lactophenol cotton blue stain. Trichophyton verrucosum was identified by examining the status of the hyphae, chlamydiospores, arthrospores, and blastospore structures under the microscope (Arda 1997).

All the animals in the groups were fed in the same environment and ad libitum (with tap water and forage from the same source).

Prior to beginning the application, the lesions of all animals in the control and the experimental group were brushed with a medium hardness brush. After that, acetate paper was placed on the lesions and the diameter of the wound was drawn on the paper. The diameters were calculated using millimeter paper (Hizli 2020). No substances were used for the control group. The extract of MS was sprayed onto the lesions of the experimental group using an atomizer. The spraying was done to cover the area where the lesions were localized and to wet them sufficiently.

This procedure was carried out twice daily (morning and evening) and during the 15 days. Starting from the pre-application (0th day) until the 36th day at intervals of two days, the lesion diameters of the cattle in the control and the experimental group were measured and recorded.

Statistical Analysis

Data from this research were statistically evaluated using the IBM SPSS 20.0 computer program. The Shapiro-Wilk test checked the normal distribution status of the data in the groups. Since the test results showed a normal distribution, the independent sample t-test was used to compare parameters between groups. The results were presented as a mean and standard deviation. In the study, $p<0.05$ was found to be statistically meaningful.

RESULTS

The lesion diameters of the control and the experimental group, which started from the first day of application (day 0) and measured at intervals of two days, are presented below in Table 1.

Table 1: Descriptive statistics of lesion diameters of the cattle groups (mm)

Day	Control Group (n=10)				Experimental Group (n=10)			
	Min.	Max.	SD	Mean	Min.	Max.	SD	Mean
0	14.00	27.00	4.58	20.10	16.00	27.00	3.82	22.20
3	14.00	27.00	4.30	20.10	16.00	27.00	3.59	22.50
6	1500	27.00	1.31	20.50	16.00	27.00	3.64	22.20
9	18.00	27.00	3.53	22.40	17.00	26.00	3.07	21.10
12	18.00	27.00	3.52	22.70	14.00	24.00	3.24	19.90
15	20.00	30.00	3.17	23.90	14.00	24.00	2.82	18.80
18	20.00	30.00	2.95	24.60	10.00	22.00	3.94	16.40
21	22.00	30.00	2.58	25.70	8.00	20.00	4.50	14.90
24	22.00	30.00	2.58	26.30	6.00	20.00	4.37	13.60
27	22.00	32.00	3.16	27.00	3.00	20.00	4.55	11.90
30	24.00	32.00	2.83	27.70	0.00	18.00	4.74	10.60
33	24.00	32.00	2.83	27.70	0.00	16.00	4.64	9.00
36	25.00	35.00	3.58	29.20	0.00	14.00	5.16	6.60

In Table 1 above, the mean diameter of the lesion prior to the application (day 0) in the control group was 20.10±4.58 mm. In this group, the mean size of the lesions increased from day 6 and reached 29.20±3.58 mm on day 36. In the experimental group, the mean diameter of the lesion prior to the application (day 0) was 22.20±3.82 mm. In the experimental group, the lesion diameter began to decrease from the 6th day and decreased to 6.60±5.16 mm on the 36th day.

Table 2 compares the lesion diameters by day in the cattle groups. Table 2 shows that there is no statistical difference between the mean lesion diameter in the control and the experimental groups before applying ($p>0.05$). At the end of the follow-up period (36th day), it was found that the mean lesion diameter in the experimental group decreased significantly ($p<0.05$) compared to the control group

Table 2: Lesion diameters of the cattle groups by the days

Days	Control Group (Mean ± SD)	Experimental Groups (Mean ± SD)	P value
Day 0	20.10±4.58 [a]	22.20±3.82 [a]	p>0.05
Day 36	29.20±3.58 [a]	6.60±5.16 [b]	p<0.01

[a,b]: Those with different letters on the same line were considered to be statistically significant in the p<0.05.

The control group lesion is presented in Figure 1, and the experimental group lesion in Figure 2 (arrows).

Figure 1: Appearance of lesions in the control group (**A:** Before treatment, **B:** After treatment)

Figure 1 above shows a fungal lesion on the back prior to the application (Figure 1A, arrow). In the control group, no signs of lesion improvement (reduced lesion diameter, hair growth, loss of colonies, crust, etc.) were observed in the examination performed by inspection during the follow-up period (Figure 1B).

Figure 2 below shows the fungal injuries in the experimental group. Growth and development of fungal lesions in the neck and shoulder blade area (arrows) were observed to stop at the end of the monitoring period (36th day). Moreover, although some lesions have completely disappeared, lessening was observed in some lesions (Figure 2B)

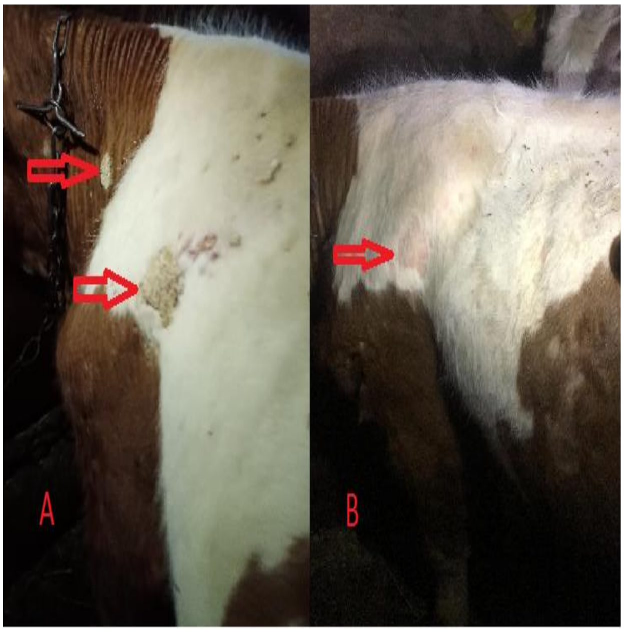

Figure 2: Appearance of lesions in the experimental group (**A:** Before treatment, **B:** After treatment)

DISCUSSION AND CONCLUSION

Ringworm disease in bovine animals is among the major diseases. The disease leads to losses of meat, milk, skin and treatment costs on farms, causing economic damage (Abdullah et al. 2021). It also threatens human health through the infection of people (Tartor et al. 2020). For this reason, the fight against disease is of great importance in terms of protecting human and animal health. Some drugs are used in the treatment of skin fungus in cattle. Griseofulvin and azoles are some of them. In the treatment of the disease, locally effective drugs are more preferred by breeders. The most important reason for this is that it is cheap and easy to apply. For this purpose, salicylic acid, benzoic acid, chlorhexidine, and iodine derivatives are most often used (Al-Farha and Mahmood 2021).

There are a lot of factors which negatively affect the fungal treatment. These include drug costs, long duration of treatment, use of low or high doses of drugs, non-use of effective drugs, antifungal resistance, drug residue in systemic treatment and various side effects (such as liver toxicity) (Hajyani et al. 2015; Jabri et al. 2017). These causes have led researchers to investigate natural, low-toxicity herbal treatment methods that can be used to treat fungi (Tartor et al. 2020).

Several studies have revealed the effects of MS. MS leaf extract has been reported to be effective against *Fusarium oxysporium*, *Rhizopus stsholonifer*, *Trichoderma sp*, *Penicillium sp* (Zohra et al. 2013), *Alternaria alternata*, *Penicillium expansum* and *Mucor piriformis* type fungi in vitro conditions (Parveen et al. 2014). Also *C. albicans*, (Cardoso et al. 2012; Alizadeh et al. 2017), *C. krusei*, and *C. tropicalis* are reported to be effective (Cardoso et al. 2012). Silver and silver chloride obtained from the leaf extract of the plant are reported to have inhibitory effects against *Bacillus subtilis*, *Pasteurella aeruginosa,* and *C. orthopsilosis* (Feizi et al. 2018). In addition to these effects, it is suggested that when administered to cancer patients before treatment with cisplatin, drug damage to the liver and kidneys is reduced, as well as anti-inflammatory and antioxidant effects (Yarijani et al. 2018).

In a study, MS is reported to increase the number of lymphocytes in mice infected with *C. albicans*, stimulating the immune system and reducing the severity of infection (Hajyani et al. 2015). In other studies, it has been observed that MS plays a protective role against oxidative stress, kidney (Saad et al. 2016), testicular and heart damage caused by Lithium carbonate applied intraperitoneal to mice (Saad et al. 2017). In addition, it is reported to be anti-inflammatory and antipsoriasis effective by reducing chronic burning edema, keratinocyte hyperproliferation and leukocyte migration on mouse ears (Prudente et al. 2017). It is reported that MS reduces paracetamol-induced liver damage (Hussain et al. 2014), kidney damage in ammonium metavanadate toxication (Marouane et al. 2011), heart muscle damage in myocardial ischemia (Zuo et al. 2017), and when applied locally on burn wound accelerates healing (Nasiri et al. 2015). It is suggested that MS stimulates the digestive tract against loperamide-induced constipation (Jabri et al. 2017), has an analgesic and anti-inflammatory effect (Seddighfar et al. 2020).

In this study, the mean diameter of the lesion prior to the application (day 0) was 20.10±4.58 mm in the control group and 22.20±3.82 mm in the experimental group. In the control and the experimental groups, the lesion diameters on day 36 was 29.20±3.58 mm and 6.60±5.16 mm, respectively (Table 1). It was observed that the size of the lesion increased over time in the control group and decreased in the experimental group. As shown in Table 2 above, the difference in the diameter of the control and treatment group lesions before application (day 0) was statistically non-significant ($p>0.05$). However, at the end of the follow-up period (day 36), the diameter of the lesion in the experimental group declined significantly ($p<0.05$) compared to the control group. In the present study, no improvement was observed in the control group at the end of the follow-up period (day 36) (Figure 1A, Figure 1B). However, in the treatment group, it was noted that while some fungal lesions were completely lost, there were signs of healing in some lesions (Figure 2B).

The results of this study are consistent with those reported by Zohra et al. (2013), Parveen et al. (2014), Alizadeh et al. (2017), Cardoso et al. (2012), and Feizi et al. (2018). This similarity may be explained by the similarity of the environmental conditions (such as temperature, humidity, oxygen, and nutrient needs) and metabolic activities required for the reproduction and development of fungal species causing dermatophytosis and other fungal species.

In this study, it was observed that MS extract applied to fungal lesions stopped the growth of lesions and stimulated healing at the end of the follow-up period (36th day). These results indicate that MS is effective against skin fungi in vivo. The results of the current study are consistent with those reported by Jabri et al. (2017), Saad et al. (2016), Saad et al. (2017), Prudente et al. (2017), Hussain et al. (2014), Marouane et al. (2011), Zuo et al. (2017), Seddighfar et al. (2020), Nasiri et al. (2015), Hajyani et al. (2015), and Yarijani et al. (2018). In the studies conducted on plants, it is reported that the Henna plant (*Lawsonia inermis Linn*) leaves are effective against bovine skin fungus (Hizli 2020), and extract of *Aloe vera* is effective against *Trichophyton verrucosum* and *Trichophyton mentagrophytes* fungi species (Tartor et al. 2020). The results of the present study are similar to those reported by Hizli (2020) and Tartor (2020). On the basis of this similarity, it can be argued that the plants *Aloe Vera*, *Lawsonia inermisLinn,* and MS contain antifungal chemicals.

The most important chemical structure of MS is made up of mucilage, flavonoids, tannins, tocopherols, ascorbic acid, carotenoids and anthocyanin (Barros et al. 2010; Hajyani et al. 2015; Jabri et al. 2017). Flavonoids have antifungal effects by inhibiting a variety of enzymes in eukaryotic cells (Cushnie and Lamb 2005).The anti-fungal effect of the MS extract, pulverized on fungal lesions, is explained by the flavonoids contained in its structure. It may be argued that flavonoids interfere with the various enzymatic systems that *Trichophyton verrucosum* requires for reproduction and development. It is also reported that MS extract stimulates the immune system by increasing the number of lymphocytes in mice infected with C. albicans (Hajyani et al. 2015). Based on this information, MS extract is thought to accelerate recovery by stimulating the immune system in cattle with trichophytes. Also, the effect of MS on lesions may be explained by the anti-inflammatory and anti-oxidant properties of MS against fungal inflammatory reactions and oxidative damage in the skin. MS is thought to have an antioxidant effect with its vitamin C content. With this antioxidant property, it can be said that it shows its effect by protecting cell structures (such as DNA, macromolecules, and cell membrane) against oxygen groups, released as a result of inflammatory reactions. Furthermore, vitamin C supports keratin tissue and stimulates collagen synthesis. These properties are considered useful in the recovery process.

After all; the aqueous extract of MS, which was sprayed on fungal lesions in cattle diagnosed with Ringworm, had a healing effect. The use of MS extract locally is recommended to treat Trichophytosis in cattle. If the current study is supported by other studies, MS extracts can be considered as a plant-based treatment option for fungal infection.

CONFLICTS OF INTEREST

The author reports no conflicts of interest.

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The study has been approved by the Ethics Committee of Kafkas University (decision date 26.01.2023 and numbered KAU-HADYEK/2023-011) and Ministry of Agriculture and Forestry of Turkey (letter dated 20.12.2022 and numbered E-29486769-325.99-8174016).

AUTHOR CONTRIBUTIONS

Idea / Concept: ED
Supervision / Consultancy: ED
Data Collection and / or Processing: ED
Analysis and / or Interpretation: ED
Writing the Article: ED
Critical Review: ED

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