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Research article
Ερευνητικό άρθρο

A study on the use of henna plant (*Lawsonia inermis Linn*) for the treatment of fungal disease (*Trichophypton verrucosum*) in calves

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ABSTRACT. The aim of this study was to investigate the usability of henna plant (*Lawsonia inermis Linn*) in the treatment of dermatophytosis lesions (*Trichophypton verrucosum*) in cattle. The animal material of the trial consisted of 50 holstein calves between the ages of 4 and 6 months, who were found to have a dermatophytosis lesion on their face and neck in their clinical examination. The experiment was organized on a three-group repeated measurement trial plan. I. Group: Trichlorfon (Neguvon 75%, Bayer) ointment, II. Group: Henna applied, and III. Group: Control Group, no treatment, and 20 (10 females, 10 males), 20 (10 females, 10 males), and 10 (5 females, 5 males) totally 50 calves used, respectively. The research was continued for 14 days until the lesions were completely healed. I. and II. Groups were observed the best healing in the calves, respectively. In the III. Group without any treatment, there was no improvement and the lesions were enlarged. In addition, the effect of gender in the treatment process of dermatophytosis lesions was insignificant. As a result of this study, it is thought that henna plant can be used in the treatment of dermatophytosis.

***Keywords*:** Dermatophytosis; *Trichophypton verrucosum*; Henna (*Lawsonia inermis Linn)*; Repeated measurement; Ringworm

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INTRODUCTION

Henna (*Lawsonia inermis Linn)* is one of the oldest and most popular plants known among medicinal plants and is a perennial shrub widely grown in North Africa (Solecki, 1975; Bhattacharjee and De 2003; Borade et al. 2011). Henna contains phenol compounds, naphthoquinone derivatives (mainly lawson), terpenoids, sterols, xanthines, aliphatic compounds, coumarins, carbohydrates, flavanoids, essential oils and other chemical components (Makhija et al. 2011; Santosh et al. 2013). Henna leaves contain a dense pigment called hennotannic acid (2-hydroxy-1,4-naphthoquinone) lawsone. This pigment has orange-red colorant properties and in studies conducted in different countries, it has been observed that naftokinone-derived dyes such as lawsone have antibacterial and antifungal activities (Jain et al. 2010; Rayavarapu et al. 2011).

It is used as an antifungal antibacterial compound since it contains *Cassia obovata* anthraquinones in henna leaf powder (Trease and Evans, 1983; Vaidya, 2000; Wallis, 2001). It has been reported that henna has antibacterial effects, antifungal activity against dermatophytes, wound healing, antitumoral effects, hypotensive, astringent and sedative effects, Henna has been reported by many researchers to have different healing effects, antibacterial effects especially for gram positive bacteria, antifungal activity against dermatophytes and wound healing (Berenji et al., 2010; Elmanama et al., 2011; Jain et al., 2010; Muhammad and Muhammad. 2005).

Dermatophytosis (Ringworm / dermatomycosis) is an infectious and zoonotic disease characterized by various lesions in many animal species and different parts of the body (Arda, 2006; Barbieri et al., 2017; Cabañes et al., 1997; Chermette et al., 2008; Sahal, 1994). *Trichophyton*, *Microsporum* and *Epidermophyton* are the three genera that cause dermatophytosis of fungal agents and there are many species within them. Many species of *Trichophyton* and *Microsporum* cause clinical infections in farm animals. The species known as *Trichophyton verrucosum* causes clinical infection especially in cattle (Yılmazer and Aslan, 2010; Şennazlı, 2018; Or and Bakırel, 2002). The infection caused by dermatophytosis in humans and animals is called “ringworm” or fungal in society (Wabacha et al., 1998; Weber, 2000).

Wagini et al., (2014) repored that together with henna, the fungitoxic effects of 13 species known as dermatophytes on 30 plant species were tested and only the full toxic effect of henna was observed. In addition, Ponugoti, (2018) repored that aqueous extract of henna leaves were tested for antifungal potential against eight important *Aspergillus* species isolated from sorghum, corn and paddy seed samples, and petroleum ether, benzene, chloroform, methanol and ethanol extract of the plant showed significant antifungal activity. Ponugoti, (2018) reported this finding suggested that henna extract may be used as an alternative source of antifungal agent for the protection of fungal infection.

Başoğlu et al., (1998), Kırmızıgül et al. (2008a, 2009b, 2013c), Cam et al., (2009) repoted that systemic antifungal and topical treatment were used for the treatment of wounds caused by dermatophytes. In a study has done with henna by Muhammed H and Muhammed S, (2005) reported that the mixture of powder henna leaves with water prevents the wound from growing in burns. Polat, (2014) reported that a stray dog that has two pecuniary lossy wound on applied a mixture of henna, butter and povidin iodee in certain proportions to the injured area and reported that the wound healed quickly.

This study was carried out to investigate the possibility of using henna in the treatment of dermatophytosis lesions in cattle.

MATERIAL AND METHODS

Ethical scope

This study was conducted in accordance with the principles of the Local Ethics Committee in the framework of the ethics confirmed by the Çukurova University Directorate of Local Ethics Committee of Animal Experiments (29.01.2018).

Fifty head Holstein Friezian calves aged between 4-6 months and with a live weight of 80-90 kg were used at the Eastern Mediterranean Agricultural Research Institute. I. Group: Trichlorfon (Neguvon 75%, Bayer) ointment was applied, II. Group: Henna slurry that obtained from the mixture of henna leaf powder with 1/2 ratio of water was applied, and III. Group, Control Group: calves was not applied any treatment, and 20 (10 females, 10 males), 20 (10 females, 10 males), and 10 (5 females, 5 males) totally 50 calves used, respectively. Calves were randomly distributed according to the wound size, and care and feeding were applied homogeneously in all Groups. In all Groups the lesions were brushed with a medium hardness brush before any treatment. For the initial

measurements of the experiment, the wound size was drawn on the acetate paper placed on the wound and then the size of the wound was calculated in mm^2 with the help of millimetric paper. In the III th Group only the lesions were measured by brushing every day, and since there was no improvement the trial was terminated on the 14th day when complete recovery was achieved in the I th, II th Groups.

Since each of the observations were obtained from the same experimental unit (calf) and included a period of time in terms of healing time. One-way analysis of variance was performed in the General Linear Model approach by the Repeated Measurement procedure in IBM SPSS 22. Differences in means between applications were compared by Tukey multiple comparison test statistic. In the analysis of variance, Mauchly's Sphericity test was used to ensure the validity of the F test. Greenhouse-Geisser, Huynh-Feldt, or Lower-bound corrections, which correct the degrees of freedom, were used to determine whether the differences between sphericity test and all dependent group combinations were equal (Box, 1954; Greenhouse and Geisser, 1959; Huynh and Feldt, 1976).

RESULTS

Mauchly's Sphericity test was applied to the F-test to be valid in one-way analysis of variance of repeated measured data and it was found to be significant at (Table 1) ($P < 0.001$).

Thus, Greenhouse-Geisser, Huynh-Feldt or Lower-bound estimates, which corrected degrees of freedom, were used to interpret the ANOVA F test and The Analysis of Variance Table is divided into two sources as Test-Between-Subjects Effects, Test-Within-Subject Effects. As seen Table 2. Test-Between-Subjects effects of the variance analysis results the difference between the groups was statistically significant ($P < 0.0001$) and the difference between the genders was not statistically significant ($P > 0.05$). On the other hand, Test-Within-Subject Effects of The variance analysis results the days (recovery time of calves) and the interaction of the days and groups were statistically significant ($P < 0.0001$) and the results are shown in Graph 1 and Graph 2.

Table 1. Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Day	0.000	1784.546	90	0.000	0.117	0.128	0.077

Table 2. Repeated Measures Variance Analysis

Tests of Between-Subjects Effects

Source		**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Intercept		290907	1	290907	225,021	0.000
Groups		102167,6	2	51083,81	39,514	0.000
Gender		168,894	1	168,894	0,131	0,719
Error		59468,83	46	1292,801		
Tests of Within-Subjects Effects						
Day	Sphericity Assumed	12341,96	13	949,381	14,602	0.000
	Greenhouse-Geisser	12341,96	1,526	8089,462	14,602	0.000
	Huynh-Feldt	12341,96	1,67	7389,194	14,602	0.000
	Lower-bound	12341,96	1	12341,96	14,602	0.000
Day * Groups	Sphericity Assumed	119904,8	26	4611,723	70,93	0.000
	Greenhouse-Geisser	119904,8	3,051	39295,45	70,93	0.000
	Huynh-Feldt	119904,8	3,341	35893,82	70,93	0.000
	Lower-bound	119904,8	2	59952,41	70,93	0.000
Day * Gender	Sphericity Assumed	341,519	13	26,271	0,404	0,969
	Greenhouse-Geisser	341,519	1,526	223,847	0,404	0,614
	Huynh-Feldt	341,519	1,67	204,469	0,404	0,632
	Lower-bound	341,519	1	341,519	0,404	0,528
Error(Day)	Sphericity Assumed	38880,62	598	65,018		
	Greenhouse-Geisser	38880,62	70,181	554,002		
	Huynh-Feldt	38880,62	76,832	506,044		
	Lower-bound	38880,62	46	845,231		

Differences between the groups were found with Tukey test statistics and the results were given in Table 3 and the best healing was seen in I th Group where Trichlorfon ointment was applied. This was followed by henna application II th Group. The mean, standard deviations, minimum and maximum values of fungal lesions according to treatment Groups and genders were given in Table 4.

Table 3. The means of trial groups comparison by Tukey test statistics

Groups	N	Mean	Std. Dev.	Std. Error
I. Group	20	7.49a	7.73	2.15
II. Group	20	16.50b	7.71	2.15
III. Group	10	40.47c	5.52	3.04

P < 0.05; I. Group: Trichlorfon; II. Grup: Henna: III. Group: Control

The results of I., II., III th Groups were used in the treatment in calves were shown in Table 4.

Table 4. Descriptive statistics of experimental groups in daily recovery process

	Female					**Research Groups**								
	III. Group					II. Group					I. Group			
	N	**Mean**	**±Ss**	**Min.**	**Mak.**	**N**	**Mean**	**±Ss**	**Min.**	**Mak.**	**Mean**	**±Ss**	**Min.**	**Mak.**
1. day	5	20.1	7.34	12.8	30.2	10	33.68	21.71	12	80	34.14	21.2	11.25	70.3
2. day	5	20.76	7.69	13.5	32	10	33.68	21.71	12	80	30.4	21.2	9.3	66.5
3. day	5	21.68	7.5	15	33	10	32.71	21.65	11	78	22.3	17.8	2	57.4
4. day	5	22.86	8.08	16	35	10	30.67	21.73	9	75	14.79	15.4	**0**	48.2
5. day	5	24.54	8.39	17.5	37	10	27.43	20.96	6	70	8.38	11.5	0	35.3
6. day	5	26.34	8.79	19	40	10	23.38	19.57	4	61.5	3.85	7.02	0	22.5
7. day	5	31.2	8.26	23	43	10	18.53	16.93	2	50	1.24	3.02	0	9.4
8. day	5	37.78	8.58	28.9	47	10	14.5	15.11	**0**	43	0.32	1.01	0	3.2
9. day	5	43.2	10.38	33	58	10	10.92	12.26	0	33	**0**	**0**	**0**	**0**
10. day	5	48.3	12.33	38	66	10	6.75	8.37	0	20	0	0	0	0
11. day	5	55.5	15.26	44	79	10	3.3	4.52	0	12	0	0	0	0
12. day	5	63	18.06	47	91	10	1.6	2.41	0	7	0	0	0	0
13. day	5	72.32	26.47	47	115	10	0.3	0.67	0	2	0	0	0	0
14. day	5	79.5	32.94	48	133	10	**0**	**0**	**0**	**0**	0	0	0	0
	Male													
	N	**Mean**	±Ss	**Min.**	**Mak.**	**N**	**Mean**	±Ss	**Min.**	**Mak.**	**Mean**	±Ss	**Min.**	**Mak.**
1. day	5	17.38	7.36	10	26.3	10	33.65	18.18	11.3	65	32.05	16.2	15	62
2. day	5	18.05	7.83	10	28.3	10	33.61	18.23	11.3	65	25.85	17.5	8.5	58
3. day	5	19.27	8.97	10.5	32	10	32.95	18.07	11	65	18.4	16.6	2	50
4. day	5	20.8	8.91	13.2	34	10	30.6	17.86	9	63	11.37	13.2	**0**	37.7
5. day	5	22.84	10.12	14.6	38.6	10	27.1	17.27	6	59	5.1	6.89	0	20
6. day	5	25.86	12.3	15.8	45	10	22.1	16.38	3	52	1.3	2.36	0	7
7. day	5	28.3	13.45	17	49.5	10	16.9	14.69	1	44	0.3	0.95	0	3
8. day	5	33.05	13.6	21	53.3	10	12	12.33	**0**	36	0	0	0	0
9. day	5	39.96	10.05	29.8	54	10	8	9.45	0	27	0	0	0	0
10. day	5	47.2	10.16	35	61	10	4.6	6.1	0	18	0	0	0	0
11. day	5	56.2	11.69	43	69	10	2.2	3.52	0	10	0	0	0	0
12. day	5	66.2	11.52	51	79	10	0.7	1.64	0	5	0	0	0	0
13. day	5	77.6	14.36	58	97	10	0.2	0.63	0	2	0	0	0	0
14. day	5	93.4	20.53	65	123	10	0	0	0	0	0	0	0	0

(P < 0.0001); Grup I: Trichlorfon; Grup II: Henna: Grup III: Control

It was exemined results that although, the mean lesion areas in both male and female calves increased in the III th Group. The healing process in the I th Group started on 2th day, the mean lesion areas continued to shrink and females recovered completely on the 9th day and, in males on the 8th day. In the II th Group, recovery started in males on the 2nd day, females on the 3rd day and finished on the 14th day completely. Also, no improvement was observed in the Group III and lesions were enlarged. Lesion size increased during the 14-day period from 20.1 ± 7.38 mm to 79.5 ± 32.94 mm in females and from 17.38 ± 7.36 mm to 93.4 ± 20.53 mm in males. In experiment, triple interaction Groups x Gender x Recovery Time is shown in Graph 1. The effects (daily change) of Grup I, II, III applications on healing time throughout the trial are shown in Graph 2.

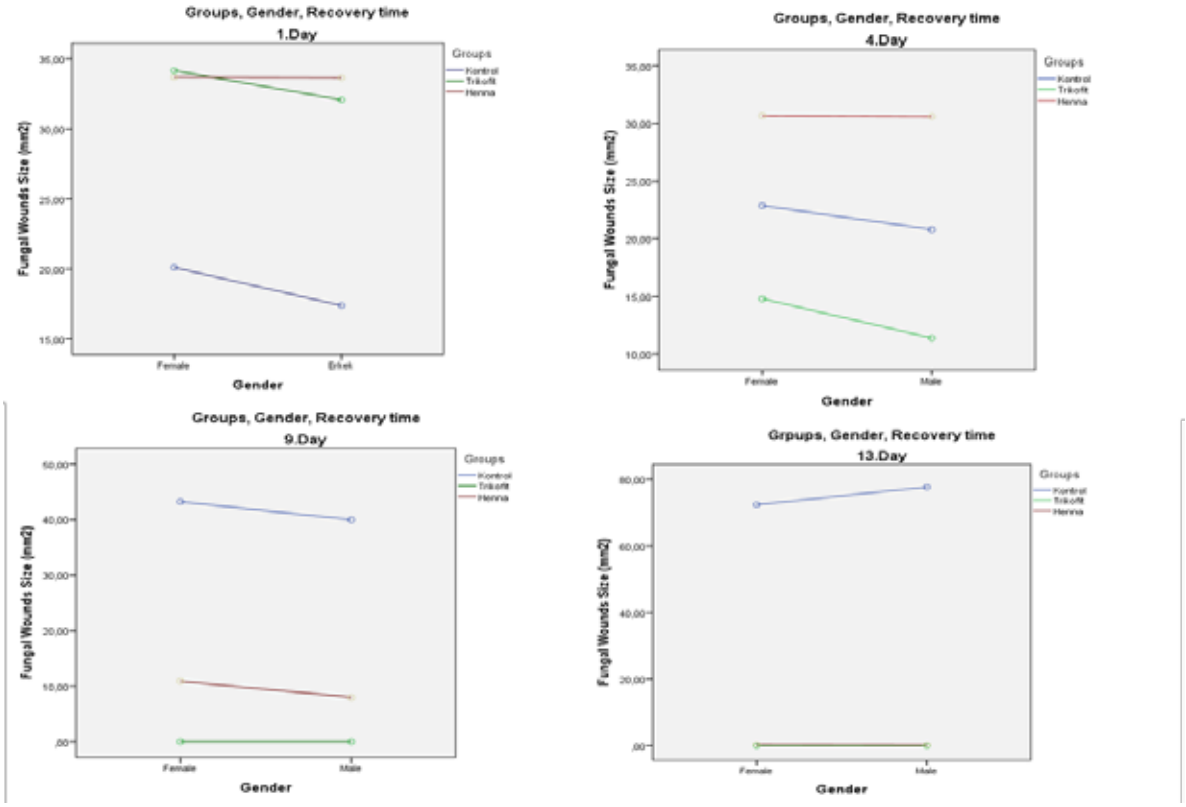

Figure 1. Change of interaction of group and gender

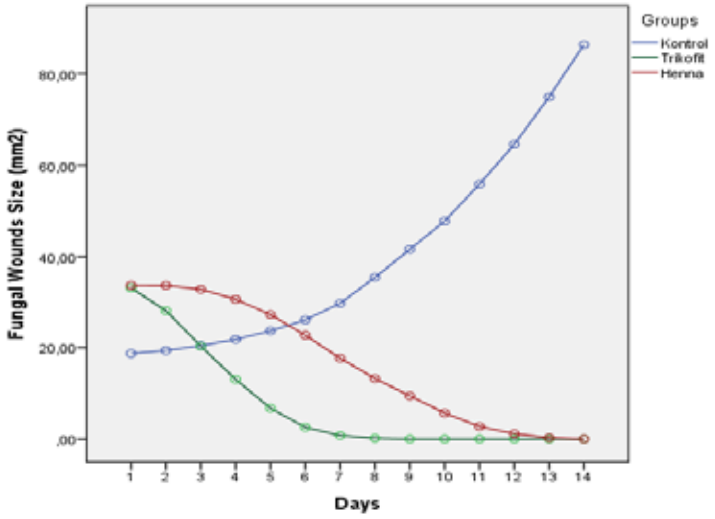

Figure 2. Distribution of the effects of groups on recovery time

DISCUSSION

The differences between the minimum and maximum values (mm^2) increase in the I. and II. Groups from the beginning of the recovery and cause the standard deviations to increase. In I th Group mean and standard deviation 8th day in females(14.5 ± 15.11), minimum and maximum values (0; 43) and, in males (12 ± 12.33) and (0; 36) as it has been realized. In II th Group mean and standard deviation 4th day in females (14.79 ± 15.4), minimum and maximum values (0; 48.2) and, in males (11.37 ± 13.2) and (0;37.7) realized. The standard deviation was never greater than the mean because the wounds in the III. Group were constantly growing. Also, Başoglu et al., (1998) reported that 57 calves with 1-6 cm ringworm in the neck area applied henna slurry four times a month and reported that all calves healing at the end of one month. This result is similar to the results of this study in that the henna completely heals the fungal wounds in calves.

The results, reflected by Yılmazer and Aslan (2010) that effectiveness of using of neguvon and whitfield's ointment in the treatment of fungal disease in cattle were in agreement with the results of the present study and the application of trichophyte ointment. Additionally, Kırmızıgül et al., (2008, 2009, 2013) reported that the healing of dermatophytosis lesions started on day 3 and ended on day 7 completely. These findings were similar to the results of the present study. In the current study, improvement starts on the 4th day and ends on the 8th day. It is thought that the longer duration of the healing is due to the different wound size and the different feeding program applied and the different environmental conditions such as climate and season. Abdulmoneim, (2007) reflected that leaf samples of *Lawsonia inermis* examined their antimicrobial potentials by phytochemical analyzes and determined the presence of anthraquinones as the main component in henna leaves. As a result, since this condition is known to have antimicrobial activity in general, it has been reported that henna leaves have antibacterial, antifungal activity and can be used in the treatment of bacterial infections. Collected henna samples from different regions of Oman have been shown to have the highest antibacterial activity against *P. aeruginosa* organisms. During screening of barks of 30 plant species against *Microsporum gypseum* and *Trichophyton mentagrophytes*, only *Lawsonia inermis Linn* extract exhibited absolute toxicity, the leaves of *L. inermis L.* were also found to exhibit strong fungi toxicity and non-phytotoxicity reported by Habbal et al. (2011). Lawsone isolated from the leaves of *L. inermis* has shown significant antifungal antibiotic effetcs reported by Santosh et al. (2013). All these stated results were similar to the results of this study Also, Rahmoun et al. (2013) stated that henna was widely used on the skin and was clearly safe to use. In addition, Yadav et al. (2013) reported that henna plant and extract against *Microsporum gypseum* and *Trichophyton mentagrophytesine fungi*, absolute toxicity, even in high temperature even if this toxicity stated that the activity continues. In addition, Kızılgül et al. (2008), Karpe et al. (2011) and Jamshidi-Kia et al. (2018) reported that dermatophytosis lesions started to heal on the 3rd day with different drugs and they completely healed on the 7th day. These findings were similar to the results of the present study.

In the present study, healing starts on the 2th day and ends on the 14th day and it is thought that the longer duration of the healing is due to different wound size and different feeding program, different environmental conditions such as climate and season.

CONCLUSION

Although the treatment of fungal disease in cattle (*Trichophypton verrucosum*) requires a slightly longer treatment period than synthetic medicine, it is thought that henna can be used instead of synthetic drugs, but further researches are needed on this subject.

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CONFLICT OF INTEREST

None declared.

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