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

H. HIZLI

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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 (*Trichophyton 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, 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; *Trichophyton verrucosum*; Henna (*Lawsonia inermis* Linn); Repeated measurement; Ringworm
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 antrarquinones 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 Muhammed, 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 Bakirel, 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) reported 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) reported 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) reported 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 iodide 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 mm² 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*

| Within Subjects Effect | Mauchly’s W | Approx. Chi-Square | df | Sig. | Epsilonb |
|------------------------|-------------|--------------------|----|------|----------|
| Day                    | 0.000       | 1784.56            | 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. Group: 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

| Gender | III. Group | II. Group | I. Group |
|--------|------------|-----------|----------|
|        | N  | Mean ±Ss | Min.     | Mak.     | N  | Mean ±Ss | Min.     | Mak.     | N  | Mean ±Ss | Min.     | Mak.     |
| Female | 1. day | 5  | 20.1 ±7.34 | 12.8     | 30.2     | 10 | 33.68 ±21.71 | 12     | 80       | 34.14 ±12.26 | 0         | 11.3     |
| Male   | 1. day | 5  | 17.38 ±7.36 | 10      | 26.3     | 10 | 33.65 ±18.18 | 11.3    | 65       | 32.05 ±16.2 | 15        | 62       |

(P < 0.0001); Grup I: Trichlorfon; Grup II: Henna; Grup III: Control
It was examined 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 (mm²) 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, Başoğlu 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 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 (Trichophyton 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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