

Effects of *Lucilia sericata* Maggot Therapy in Chronic Wound Treatment: A Randomized Clinical Trial

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Background: Chronic ulcers are one of the challenges of treatment today and cost a lot to the health system. The present study was conducted to investigate the effect of *Lucilia sericata* maggot therapy in chronic wound treatment.

Materials and Methods: This study was conducted as a clinical trial study with 90 eligible patients. Patients were randomly assigned to two equal intervention and control groups. Both groups received routine treatments for chronic ulcers. Patients in the intervention group received maggot therapy with larvae of *Lucilia sericata*. For all patients, a smear and culture of wound discharges were acquired. The condition of wound healing, the type of infection, and the reduction of microorganisms were compared between the two groups.

Results: *Staphylococcus aureus* was present in 68.9% of the patients and was the most abundant infection among all patients. Results of culturing after larval treatment at different times revealed a decrease in the number of all bacteria, especially *Pseudomonas aeruginosa*, *Escherichia coli*, and *S. aureus*, but the response rate for *Enterococcus* was the lowest. Also, the wound healing rate and reduction in necrotic tissue at the end of the second week ($p=0.041$) and the third week ($p=0.012$) was significantly higher in the intervention group.

Conclusion: Larvae of *L. sericata* have the highest effects on *P. aeruginosa* and had the least effect on the growth of *Enterococcus*. Also, our results showed larvae of *L. sericata* therapy can significantly improve wound healing rate.

Keywords: chronic ulcers, maggot therapy, larvae of *Lucilia sericata*, wound healing

Introduction

As populations become older, the number of patients suffering from ulcers due to chronic diseases, such as pressure ulcers, diabetic and vascular ulcers, is increasing.¹ Chronic wound is a wound whose recovery requires a long time or it happens commonly and recurring which normally reduces the quality of life.² Wound infection is usually caused by exogenous microorganisms in the air and polymicrobial flora, including aerobics, facultative anaerobes, and obligates anaerobes, *Staphylococcus*, *Enterococcus*, *Corynebacterium*, and *Enterobacteriaceae* species. *Escherichia coli* is one of the common causes of chronic wound infections. Various studies have shown the production of beta-lactamase enzymes by significant number of *Enterobacteriaceae* family, increasing methicillin-resistant *Staphylococcus aureus* (MRSA) infections, and bacterial biofilm formation. Thus, it is necessary to find alternative therapies for antibiotics or assisting in treatment, antibiotics.^{1,2} One of the most important and effective factors in wound healing is

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the provision of the substrate and the conditions for recovery. These conditions include debridement of dead tissues, prevention, and control of infections and inflammation, and equilibrium in the amount of moisture in the wound.³ Wound debridement is one of the most important stages in the treatment of ulcers, especially resisting ulcers.^{2,3} Debridement includes the removal of necrotic tissues and secretions, foreign matter, and bacteria.¹ There are several methods for debridement, which include: surgical, mechanical, chemical, autolysis, and biological methods.⁴ Different studies have been done on different methods of debridement, but no study has demonstrated the superiority of a method to other methods.⁵ Debridement by larva as a biological method for the first time in 1550 by Dr. William Baer was used to improve the wounds of the army soldiers and later used for sterilization in controlled conditions.⁶

Along with increasing resistance to penicillin and other antibiotic groups and the failure of routine therapies, the use of larval care has been considered.⁷ Larva therapy is used in open wounds and wounds containing gangrene and necrotic tissues, with or without infection.^{8,9} Larva therapy stimulates wound healing and reduces bacterial load.¹⁰ So far, various studies have examined the clinical effects of larval treatment but they have reported different results.^{11,12} Given the above, the purpose of this study was to investigate the effect of Maggot of *Lucilia sericata* therapy in chronic wound treatment.

Materials and Methods

This triple-blind clinical trial was performed on 90 patients with chronic ulcers. The study was conducted on referred patients of two educational hospitals (Imam Hossain and Khatam-AL-Anbia hospitals) in Shahroud, Iran, from June 2018 to August 2018. The enrolled patients were randomly allocated to intervention and control groups.

Inclusion criteria: Having a chronic ulcer over 3 months on the outside of the body; no use of antibiotics in the last 3 weeks; no history of surgery in the desired position; no need for anticoagulants and steroids drugs; no addiction to alcohol or drugs; satisfaction to participate in study.

Exclusion criteria: Use of radiation and phototherapy in a recent month; suffering to severe diseases such as heart, liver, and kidney disease; having plenty of pain during treatment; having an uncontrollable fever during treatment. All patients were examined for necrotic tissue before splitting into the intervention and control groups and patients with deep and thick (over 70% of wound bed)

necrotic tissue requiring surgery to clear necrotic tissue were excluded. So, all patients were vascular examined by Doppler ultrasound and those with severe vascular failure were excluded.

Blinding description: In this study, patients, and the person responsible for wound healing assessment and analyzer were blinded.

The assignment of patients into two groups of intervention and control was done by a qualified nurse according to the list of assignment order which was developed by statistic consultant. Assessment of wounds was done by a qualified nurse without any knowledge of the type of intervention.

For all of the wounds in both groups, a smear sample and culture were prepared, and the type of infection was determined. Both groups received routine treatment of chronic ulcers. Routine treatment includes topical and systemic treatment, including regular wound healing, debridement, wet dressing, nutritional support, and antibiotic treatment. Before intervention phase, primary wound evaluation was done and damaged tissues were removed from wounds. Patients in the intervention group received larva therapy in addition to routine therapies. However, patients in the control group were treated with routine methods only. The number of larvae per wound varies depending on the extent and depth of the wound and the degree of infection in the various parts of the damaged site at the site of the wound, but usually, be laid 8 to 10 larvae per cm² of ulcer. Larvae should usually be removed within 48–72 hours after prescription.

Measured patient-related variables included age, sex, smoking, substance abuse and the duration of the ulcer. The variables related to the ulcer included type of ulcer, site of ulcer, wound depth (Grade II (i.e. dermal and epidermis) and Grade III involvement (i.e. joint involvement and tendon)), septic arthritis, and type of bacteria. Two times a week, the site was replaced by new larvae and the rate of necrotic tissue and new granulation tissue was evaluated and measured. In this study, the main outcome variables were removing necrotic tissues and appearing of granulated tissues. All treatments were performed for patients in both groups for at least three weeks and maximum until complete healing of the wounds. After larval treatment, the wounds were re-cultured by standard methods. The flow chart of the study is shown in [Figure 1](#).

Data analysis was performed based on intention to treat analysis by statistical software SPSS version 16. For describing the findings, descriptive statistics such as mean± standard deviation and frequency were used. Due to the quantitative

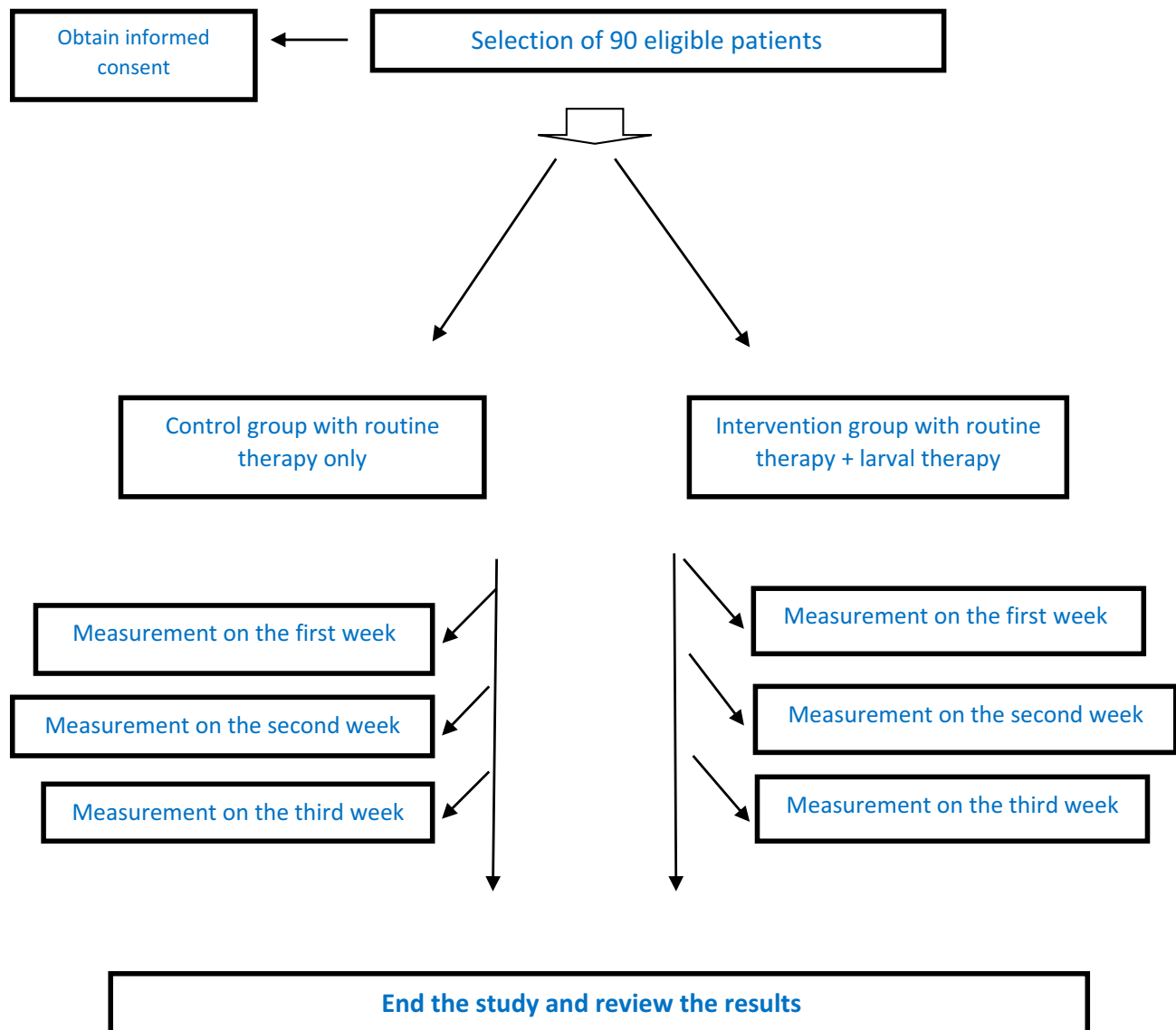


Figure 1 Flow diagram of study.

and qualitative variables, independent T and Chi-square tests were used in the significance level of $P < 0.05$.

The protocol of this study has been approved by the ethics committee of Islamic Azad University, Shahrood Branch (IR. IAU.SHAHROOD.REC.1396.53). This study has been registered in Iranian of clinical trials registration system (IRCT20100102002954N13). The essential information and the objectives of the study were explained to the patients, and a written informed consent was obtained for participation in the study.

Results

From all patients, 67 patients (74.9%) were male and the rest were female. The mean age of all patients was 55.4 ± 11.6

years (45–66 years). The mean duration of the disease was 3.7 months for chronic ulcers. Eighty-three percent of patients had lower limb ulcer and 17% had wound in the sacrum area. The demographic and clinical history data of patients in the two groups are presented in Table 1. So during the study, none of the patients needed urgent amputations and there was no case of sepsis during treatment; therefore, none of the patients were excluded from the study.

The results of culture before treatment in patients with chronic ulcers are shown in Table 2. As can be seen, *S. aureus* with 62 cases (68.9%) was the most abundant infection among all patients.

Results of culturing after larval treatment at different times revealed a decrease in the number of all bacteria, but

Table 1 Comparison of Demographic Data and Clinical Variables in Intervention and Control Groups

Variables	Intervention Group N=45	Control Group N=45	p value
Mean age (years)	56.1 ± 10.6	55.2 ± 11.8	0.088
Sex			
Male	35 (77.8%)	32 (71.1%)	0.091
Female	10 (22.2%)	13 (28.9%)	
BMI (kg/m ²)	25.6 ± 4.8	25.1 ± 5.3	0.156
Cause ulcer			
Diabetic foot	32 (71.1%)	34 (75.6%)	0.102
Bedsore	13 (28.9%)	11 (24.4%)	
History of smoking			
Yes	29 (64.4%)	31 (68.9%)	0.083
No	16 (35.6%)	14 (31.1%)	
History of addiction			
Yes	15 (33.3%)	14 (31.1%)	0.108
No	30 (66.7%)	31 (68.9%)	
Mean duration of ulcer (month)	4.2 ± 1.7	4.3 ± 1.9	0.128
Location of ulcer			
Lower extremity	38 (83.9%)	37 (82.2%)	0.148
Sacrum	7 (16.1%)	8 (17.8%)	
Depth of wound			
Grade II	6 (13.3%)	8 (17.8%)	0.077
Grade III	39 (86.7%)	37 (82.2%)	
Septic arthritis			
Positive	6 (13.3%)	5 (11.1%)	0.123
Negative	39 (86.7%)	40 (88.9%)	

Table 2 Culture Characteristics Before Treatment in Intervention and Control Groups

Bacteria	Intervention Group N=45	Control Group N=45	Total N=90	p value
<i>Staphylococcus aureus</i>	33 (73.3%)	29 (64.4%)	62 (70.0%)	0.073
<i>Pseudomonas aeruginosa</i>	1 (1.1%)	4 (8.9%)	5 (5.6%)	
<i>Klebsiella</i> spp.	2 (4.4%)	2 (4.4%)	4 (4.4%)	
<i>Escherichia coli</i>	3 (6.7%)	7 (15.6%)	10 (11.1%)	
<i>Enterococcus</i> spp.	4 (8.9%)	2 (4.4%)	6 (6.7%)	
Other	2 (4.4%)	1 (1.1%)	3 (3.3%)	
Total	45 (100.0%)	45 (100.0%)	90 (100.0%)	

the response rate for *enterococcus* was the lowest, which could indicate the resistance of this bacterium to treatment. The complete results of wound culture at different times are shown in Table 3.

The wound healing rate and removing necrotic tissues was not significantly different between the two groups at the end of the first week of study ($p=0.103$). The results of study showed significant differences in wound healing rate and reduction of necrotic tissues between two groups at the second and third weeks after intervention. The wound healing rates after treatment at different times in intervention and control groups are shown in Table 4.

Discussion

The results of this study demonstrate the use of larva in the treatment of chronic wounds could significantly reduce bacterial load/bioburden and improve wound healing. In the third week after treatment, decrees of bacteria were significantly higher compared to the control group. Also, the results of this study showed that the effect of larva therapy on some microbial strains such as *Pseudomonas aeruginosa* and *E. coli* is very good and on some microbial strains such as *Enterobacter* is relatively weak. Our findings are consistent with the results of studies by Kerridge et al and Renner et al.^{11,12}

The treatment of wounds or chronic injuries is usually slow and hard, especially if routine treatments are used. These wounds are seen in most people with poor health status.¹³ The use of larval treatment has been reported more than 100 years ago. Previous studies have emphasized the importance of using Larva therapy as an effective way to treat chronic ulcers. Larval treatment reduces the risk of amputation, reduces the use of antibiotics, prevents long-term hospitalization, and reduces the incidence of outpatient visits.¹⁴ Larval treatment can be used as the first-line treatment in patients with antibiotic resistance, infectious ulcers, immunosuppression, and diabetic disease.^{13,14} Several studies, either in the form of a clinical trial or as a systematic review, have examined the effect of larval treatment on different patients group and different aspects.^{15–17}

Valachová et al, in their study, showed that larval treatment is more effective than conventional treatments in debridement of chronic ulcers that is consistent with the results of our study.¹⁸

Table 3 Culture Characteristics After Treatment at Different Times in Intervention and Control Groups

Bacteria	Intervention Group N=45	Control Group N=45	p value*
<i>Staphylococcus aureus</i>			
First week	29 (85.3%)	26 (89.7%)	0.142
Second week	18 (52.9%)	20 (68.9%)	0.055
Third week	11 (32.4%)	15 (51.7%)	0.019
<i>Pseudomonas aeruginosa</i>			
First week	1 (100.0%)	4 (100.0%)	0.155
Second week	1 (100.0%)	3 (75.0%)	0.067
Third week	0 (0.0%)	2 (50.0%)	0.023
<i>Klebsiella</i> spp.			
First week	2 (100.0%)	2 (100.0%)	0.139
Second week	0 (0.0%)	2 (100.0%)	0.001
Third week	0 (0.0%)	0 (0.0%)	0.151
<i>Escherichia coli</i>			
First week	3 (100.0%)	7 (100.0%)	0.147
Second week	2 (66.7%)	5 (71.4%)	0.062
Third week	1 (33.3%)	3 (42.9%)	0.047
<i>Enterococcus</i> spp.			
First week	4 (100.0%)	2 (100.0%)	0.128
Second week	2 (50.0%)	2 (100.0%)	0.213
Third week	1 (25.0%)	1 (50.0%)	0.198
Other			
First week	2 (100.0%)	1 (100.0%)	0.159
Second week	1 (50.0%)	1 (100.0%)	0.028
Third week	1 (50.0%)	1 (100.0%)	0.028

Note: *Boldface indicates values that are significant ($p < 0.05$).

Table 4 The Wound Healing Rate After Treatment at Different Times in Intervention and Control Groups

Treatment	The Wound Healing Rate and Removing Necrotic Tissues		p value*
	Intervention Groups (%)	Control Groups (%)	
First week			
Diabetic foot	18%	15%	0.103
Bedsore	15%	11%	
Second week			
Diabetic foot	47%	23%	0.041
Bedsore	41%	21%	
Third week			
Diabetic foot	75%	39%	0.012
Bedsore	66%	37%	

Note: *Boldface indicates values that are significant ($p < 0.05$).

Lepage et al, in their clinical trial study, investigated the effect of larval treatment in comparison with hydrogel in wound healing and increasing of quality-adjusted life years (QALYs), which did not show any difference between the two groups. No antibiotic was used in this study. Only changes in the wound culture were investigated. The difference between the results of this study and the present study can be attributed to the difference in the technique of the two studies and the antibiotics used in this study that probably using antibiotics will increase the speed of recovery and also eliminate the necrotic parts faster.¹⁹

Morgan and Nigam evaluated the effect of larval treatment on bacterial load and resistance to *S. aureus* and observed that larval treatment is not more effective than hydrogel on reducing bacterial load but is more effective in eradicating *MRSA*. The results of this study are largely consistent with our findings and the reason for the minor differences between the two studies can be attributed to the difference in the sample size of the two studies.²⁰

In terms of the depth of involvement in our study, 85% of patients had deep ulcer (Grade III) and only 10% of patients had Grade II ulcers. In our study, 59.9% of patients had septic arthritis and 90% had infectious ulcers while in Telford et al, 56.4% had deep ulcers (Grade III), 43.6% had Grade II ulcers and 11.1% of patients had septic arthritis. The comparison of results showed that septic arthritis was more prevalent in our study. The difference between the results of this study and the present study may be due to the choice of the type of chronic ulcer and the mean age difference between the two groups.²¹

In the study of Blueman and Bousfield, the frequency of smoking was 23% in control group and 14% in larval treatment group, while in our study 63.3% of patients were smokers. Using cigarettes and opium can slowdown wound healing and prolong the course of treatment. Therefore, in the treatment of chronic ulcers, the condition of the patient and especially the use of cigarette as a confounding factor for wound healing should always be considered.²²

The important point in our study was the examination of ulcers caused by cultures and smears that have not been addressed in other studies. The results of this study showed that after treatment with larvae, there was a significant decrease in bacterial growth rate in consecutive weeks after intervention and that the highest decreasing rate of bacterial growth in smear and culture was observed in the third week after intervention (*P. aeruginosa* completely

decreased and *E. coli* decreased up to 50%). Our study results showed a maximum capacity for larval treatment in preventing the growth of bacteria which is consistent with the results of the Gilead et al study.²³

Larvae had a lower effect on reducing the growth of enterococci, in spite of the decline in the growth rate of all bacterial species in the second week, enterococci resistance showed the larvae in the second week. So in the third week, there was only a 25% drop in growth, so that growth rate of enterococci from 75% in the first week to 25% in the third week.²⁴

Limitations

One of the important limitations of this research was the failure of some patients to use larvae in their treatment, which was justified by patients. Also the relatively long period of treatment and high cost were other limitations that were solved with the help of the research deputy of Islamic Azad University of Shahrood.

Conclusion

Larvae therapy has a variety of effects on different bacterial species that have the highest effects on *P. aeruginosa*, *E. coli*, and *S. aureus* and had the least effect on the growth of *Enterococcus*. Therefore, it is suggested that more extensive studies on the number of patients should be done in multicenter studies on the effects of larval treatment on bacterial growth in chronic ulcers.

Abbreviations

QALYs, Quality-adjusted life years; MRSA, Methicillin-resistant *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *L. sericata*, *Lucilia sericata*.

Data Sharing Statement

The dataset used and/or analyzed during the present study is available from the corresponding author upon reasonable request.

Ethics, Consent, and Registration

This study has an ethics code number (IR.IAU.SHAHROOD.REC.1396.53) from the ethics committee of Islamic Azad University of Shahrood Branch and has been registered in Iranian clinical trials system with code IRCT20100102002954N13. The essential information and the objectives of the study were explained to the patients,

and written informed consent was obtained for all participants for participation in the study.

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Author Contributions

All authors of this article have played an active role in all aspects of the preparation of the article, including: design, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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