

## Topical Effect of *Mentha pulegium* Essential Oil on Burn Wounds in Wistar rats

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### ABSTRACT

**Introduction:** Burn wound is a suitable place for the development of drug resistant infections. Therefore, investigations for effective drug development have a priority. The aim of this study was to identify the effects of herbal material on infectious burn wounds in rat.

**Materials and Methods:** *Mentha pulegium* essential oil was prepared by a Clevenger apparatus. Sensitivity of clinical strain of *Pseudomonas aeruginosa* was determined by disk diffusion and microbroth dilution broth against the *Mentha pulegium* essential oil. Twenty-four Wistar rats were anesthetized by a mixture of ketamine and xylazine. Back of the rats were shaved and were burned by a molten metal. The fresh bacterial culture was inoculated on burn wounds by a cotton swap. Rats were divided into 3 equal groups. The first group was treated with essential oil, the second with silver nitrate ointment and the third group had no treatment. The wounds were evaluated for 25 days by calculating the area of the wound and microscopic examinations for infection symptoms, inflammation and histological examination.

**Results:** Minimum inhibitory concentration of diluted essential oil was 5 mg/ml. In the treatment group with silver, many bacterial cells and inflammation signs were detected. In this group the rate of wound healing was low and due to inflammation it was not easily debrided. In the control group, the scar was formed without treatment, but the recovery rate was very low and the inflammation was proven. In both these groups, *Pseudomonas* was isolated successfully. Three rats in the silver group and all of rats in control group were dead during the test. In the test group with essential oil, the least inflammation and infection was observed. Culture result of the wound was *Pseudomonas* negative. The area of the wound in test group that treated by essential oil was 86.32% on 10<sup>th</sup> day and 100% on 14<sup>th</sup> day. In the Silver group, the area of the wound was 36.124% on day 10 and 6.97% in the control group.

**Conclusion:** It was concluded that, *Mentha pulegium* essential oil was able to control the infection and improve burn wound healing in Wistar rats. This material is recommended for the clinical trials on human.

**Key words:** *Mentha pulegium*, Essential oil, Burn wound, *Pseudomonas*, Rat

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### INTRODUCTION

A burn is classified as thickness or degree (damage to all skin layers) [1]. Each year, nearly 2.4 million burns occur worldwide, with 650,000 people needed treatment [2]. Incidence and mortality rates are different for different burn injuries, depending on the area, depth, level of burn and treatment.

Burn wound healing is divided into three groups of events: inflammation phase, proliferation phase and restructuring phase. Any material that can shorten the time of these phases leads to faster repairs [3].

The rate of wound healing in skin burns is very important in terms of the costs of hospitalization, health system and for patient and his family. Any method that could reduce the recovery time will also reduce the burden of financial and psychological costs imposed on patients and their relatives. There are many methods for the topical treatment of burn wounds such as topical silver sulfadiazine, silver nitrate and biological coatings [4].

There is not enough evidence to show the effect of silver ointment on controlling burn wound infections. The present evidence emphasizes that topical silver sulfadiazine can cause an increase in infection of burn wound comparing to with dressings or other skin substitutes. This is unclear or with high risk of bias [5].

The antimicrobial effects of herbal materials on gram negative and positive bacteria have been confirmed in many studies and in many cases have been reported in conjunction with the presence of active components of monoterpenes [6-8].

Since ancient times, *Mentha* air organs have been used as a powerful disinfectant. *Mentha pulegium* has anti-pain, anti-adhesion, platelet, anesthetics, antibacterial, anti-cancer, anti-inflammatory, anti-mutagenic, anti-oxidant, anti-rheumatic, anti-spasmodic, anti-virus, astringent, anti-Candida, anti-bloating, depression Moore, urinating, gastric tincture, muscle relaxant and cyclooxygenase enzyme inhibitor [9-12].

The present study was conducted to investigate the effects of *Mentha pulegium* essential oil as a novel antibacterial agent in burn wound healing.

## MATERIALS AND METHODS

### Herbal material preparation

Essential oil of *Mentha pulegium* plant was extracted with a Clevenger apparatus by boiling, condensing and decantation. Essential oil was diluted by a suitable emulsifier.

### Bacterial strain and antibacterial assay

A previously determined drug resistance clinical strain of *Pseudomonas aeruginosa* was obtained from Infectious Diseases Research Center (IDRC) of Arak University of Medical Sciences, Iran. Susceptibility tests as disk diffusion and microbroth dilution methods were performed for diluted *Mentha pulegium* essential oil against *P. aeruginosa* as described earlier [13].

### Animal experiments

Twenty four Wistar male rats were obtained from IDRC of Arak University of Medical Sciences, Iran. Anesthetic procedure was performed by intraperitoneal (IP) injection of 100 mg/kg ketamine and 20 mg/kg xylazine. Back of the rats were shaved and the region was completely disinfected. One cm<sup>2</sup> of this area was burned by a metallic element. 0.5 milliliter of fresh *P. aeruginosa* culture with concentration of 10<sup>6</sup> CFU/ml was completely inoculated on the burn wounds.

Rats were divided into three groups in separated cages. The first group was treated with diluted essential oil as test group. The second group received silver sulfadiazine 1% ointment and control group without any treatment. The debridement process and the clinical signs of infection were assayed until the wound was fully granulated.

Treatment was started 24 hours after burning and inoculation by *P. aeruginosa*. Experiments were done in same condition by one person and continued until the closure of the wounds.

Diluted *Mentha pulegium* essential oil as 10% of crude essential oil was used for test group by spraying entire surface of the wound by 50 microliter per cm<sup>2</sup>. The control group was not received any treatment. Wounds in all groups were open and have not any dressing in order to increase visibility of wound conditions.

After scrounging the necrotic tissue into the wounds, the groups were subjected to debridement and the treatment was continued. Subsequently, bacterial culture from the scar tissue was prepared on EMB (Difco) and Blood Agar for microbiological evaluation as well as White blood cells. Wound area measurement was carried out on days 3,7,10,14,18,22 and 25.

### Pathological examination

In the last week of experiments, samples were collected in 10% formalin for histology examination at pathology laboratory.

## RESULTS

### Susceptibility testing results

In disk diffusion method it was shown that 0.312 mg/ml of diluted material could be inhibited *P. aeruginosa* by a 14 mm zone of inhibition. In microbroth dilution method 5 mg/ml was obtained as MIC.

### Animal experiments results

In the test group the wound was granulated and ready for debridement. Surface of the wound was rapidly constructed, so that the scars were clearly highlighted (Figures 1A-1C). Wounds were healed in less than 10 day and scar formed without any inflammation. Quality and quantity of infection of the burn wound versus tissue repairing speed and wound healing are directly related. A construction in the area of the wound was shown (Figures 2A-2C).

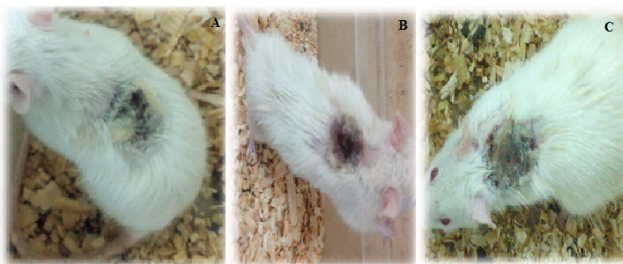


Figure 1: (A-C) The state of burn wounds before debris and due to the essential oil effect

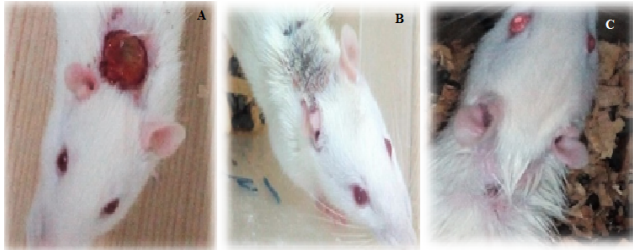


Figure 2: (A-C) The recovery condition and healing status of burns after debris due to the use of essential oil

In the second group (treated by Silver), many bacterial cell were seen. Wound healing rate was very low and the scar was not being debride-able.

In the control group scar was not formed. Wound area at beginning and end of the process has not significantly changed. Debridement was not performed because the wounds did not have debridement conditions. Granulation was very poor and scarring remained with the same initial conditions (Table 1).

Table 1: Healing status for 3 groups in days of experiment (%) (Three rats died in Silver group and all of rats in control group until 10<sup>th</sup> days)

Groups	Days of experiment						
	3 <sup>th</sup>	7 <sup>th</sup>	10 <sup>th</sup>	14 <sup>th</sup>	18 <sup>th</sup>	22 <sup>th</sup>	25 <sup>th</sup>
Essential oil	19.96	37.44	86.32	100	100	100	100
Silver	3.421	18.31	36.12	54.91	60.11	79.96	100
Control	1.2	2.3	6.97	0	0	0	0

### Microbiological examinations

Infection of wounds by *P. aeruginosa* was proved by cultural and microscopic examinations of wound secretions. Bacterial cell ratio to epithelial cells and Inflammation were seen in Gram stain slides of control and silver groups. Bacterial cells were significantly fewer than in test group (Figure 3).

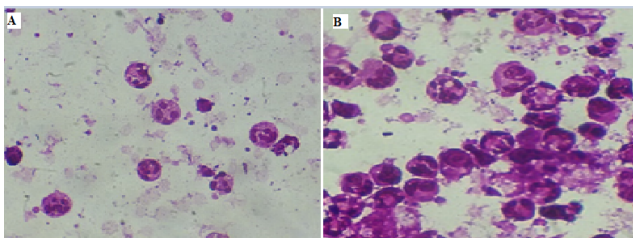


Figure 3: (A) Treated by essential oil, (B) Wound secretions treated with silver

Wound surface after debridement was quite pink and had a small discharge in the test group, but the silver group had a high level of inflammation and infectious signs.

In all groups, presence of pseudomonas in wounds was also investigated by culturing method.

Three rats in silver group and all rats in control group were dead till 10<sup>th</sup> day. All rats in test group were live and health to end of experiment at 25<sup>th</sup> day.

### Histological findings

The results of histological and studies show that there are more white blood cells and inflammation in the wound in silver group,

In test group the granulation was better and inflammation and WBC were at minimum. Restoration and skin surfaces were significantly better (Figure 4).

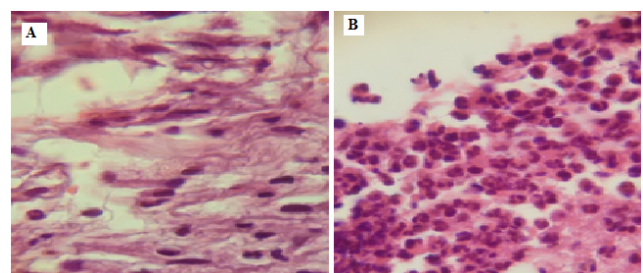


Figure 4: (A) Treated by essential oil, (B) Wound sample treated with silver

### DISCUSSION

Results of this study indicated that *Mentha pulegium* essential oil successfully could control burn wound infections and consequently accelerated wound healing.

There are four major phases in wound healing. During the final stage of wound healing, the germinated tissue is re-formed and shaped to increase the strength and tensile strength of the wound [14]. In the present study it's seems that the *Mentha pulegium* essential oil can accelerated all of the wound healing processes. There is a serious need to develop further experiments.

Pajohi et al. [15] performed the determination of the chemical composition and antimicrobial effects of Mentha essential oil on *Staphylococcus aureus*. In their experimental study, the chemical compounds of Mentha essential oil were investigated using mass spectrometry gas chromatography method, its antibacterial activity by determination of MIC of *Staphylococcus aureus* by microdilution method and morphological changes and cell membrane were evaluated using Transmission electron microscopy. MIC of *Staphylococcus aureus* was in the range of 75-1200 µg/ml.

This study showed that Mentha essential oil has very high antimicrobial potential, so it can be used in

combination with other preservatives to protect food against microorganisms causing infection and poisoning [15].

The results of this study are consistent with the previous studies. It also indicates that the essential oil has antibacterial effects by preventing the growth and interaction of bacteria.

Several studies have reported the effect of the extract of medicinal plants or natural substances on the process of skin wound healing that are agree with our research findings.

Furthermore, it has been reported that Manna topical ointment and Aloe-vera gel have the same effect on skin repair when applied locally to skin lesions.

Also, topical use of chamomile hydroalcoholic extract accelerates burn wound healing in rat [16].

### CONCLUSION

It was concluded that *Mentha pulegium* essential oil had a significant antibacterial effects and consequently accelerate the healing of burn wounds in rats. Therefore, this material is recommended for the clinical trials on human.

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### CONFLICT OF INTEREST

The authors declared no conflicts of interests.

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