Effect of Electromagnetic Form of *Melilotus Officinalis* Extract on Dermal Wound Healing in Diabetic Mice

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Summary

Diabetic foot ulcer is one of the major complications of diabetes which needs more attention. Diabetic foot ulcers are sub-classified as chronic wounds which their healing is problematic and its pathology backs to inflammatory, neuropathy, and circulation disturbances. There is no obvious cure for diabetic foot ulcer. *Melilotus officinalis* (MA) is one of the valuable plants that has been known for its potential in improvement of inflammation and microcirculation in the injured limbs. MA extract has been recently introduced with positive safety and efficacy in diabetic foot ulcers and pressure ulcers. The aim of this study was to evaluate efficacy of electromagnetically-processed MA extract (MAE) on wound healing in murine model of diabetes.

This study was conducted on 36 mice, which divided into diabetics and healthy groups. MAE was administered in two forms: treated with electromagnetic field and non-treated and then administered on wounds as a dressing.

Concerning wound size, application of both forms of MAE caused significant wound size reduction after 15 days alongside with histopathologic confirmatory results but the wound healing effects of electromagnetically-processed product was more evident.

Interestingly, the present study confirmed that MAE provides full wound healing with accelerated wound closure, and unexpectedly it improves the quality of the tissue in the healing wound with very efficient hair growth on the scars. Electromagnetic process of MAE showed its brilliant efficacy on diabetic ulcer in mice.

Key words: *Melilotus officinalis*, diabetic ulcer, diabetes, electromagnetic, antioxidants

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Introduction

One of the major complications of diabetes is diabetic ulcer that mostly seen in foot and leads to amputation remaining still a major concern in medicine. Poor control of blood glucose levels in diabetes is the major risk factor for neuropathic and vascular changes. Diabetic ulcers if not treated properly may further be complicated by infection and gangrene. Some new remedies such as oxygen chamber exposure, use of platelet-derived growth factor (PDGF), and various local dressings have shown relative efficacy but no one has been found suitable yet[1].

Some beneficial effects have been previously reported for *Melilotus* species such as improvement of venous blood circulation[2,3], reduction of inflammation[4,5,6], improvement of lymphedema[7,8], and improvement of immune system [9].

Melilotus officinalis (MA) has been recently introduced as a new drug by trade name of ANGIPARS. Preclinical acute and subchronic studies in rodents and dogs and also in vitro studies in cells confirmed its safety[10,11,12]. Results of phases I, II, and III clinical studies on ANGIPARS indicated its safety and efficacy in human diabetic foot ulcer [13,14,15,16,17,18].

In the present study, efficacies of electromagnetically processed and non-processed MAE were tested in mouse model of diabetic ulcer.

Methods

MAE in two forms of electromagnetically processed and non-processed was obtained from ParsRoos Company (Tehran). The electromagnetic field is a pulsed field at a frequency of about 250 MHZ with power of 45 watt and magnetic field of strength of 150 AμTesla. Mice weighing 30-40 g were obtained from animal house of TUMS. A total of 36 mice were used with equal numbers being assigned to each of six groups (three test groups and three control groups). Animals were caged in separate cages.

Diabetes was induced by intraperitoneally administration of streptozotocin (Sigma-Aldrich, UK) at the dose of 45 mg/kg. Blood glucose level was measured at the baseline, 48 hours after streptozocin, and every 5 days. The induction of diabetes was confirmed if the blood glucose level doubled. Blood glucose level was determined by a Glucometer (EasyGluco, USA). Regarding the entity of streptozotocin-induced diabetes, the loosing weight and week animals, and those with uncertain blood glucose levels were excluded from the study. On the first day, a full-thickness, circular 15 mm diameter wound was created using surgical razor[19]. Two forms of MAE (processed and non-processed with pulsed electromagnetic field) was given to diabetic and non-diabetic mice as test groups. Normal saline was used as control. Everyday wounds were redressed with control or test compounds. The wounds were flushed by sterile saline to remove debris and to clean the wound area. The pictures of the wound which were taken by a digital camera were evaluated for wound healing in terms of wound size and appearance of new fresh epithelium. Control of bias was achieved by assigning a code to each of the experimental groups. Investigators were blinded to the identity of each groups and the test and control had a similar appearance.

One animal from each group was euthanized at the end of the study and wounds were excised at the time of euthanasia with about 2 mm of tissue surrounding the wound, fixed in streck tissue fixative and embedded in paraffin. Histopathology images were prepared by H&E from sections of derm and epiderm. Images were examined under microscope and scored on their healing improvement by an expert histopathologist.

Statistical Analysis

All data were analysed by SPSS, version 11.5. On-way ANOVA followed by Tukey's posthoc test was used to evaluate changes between groups. A *P*-value of less than 0.05 was considered significant.

Results

Wound size and results of histopathology examinations are shown in Table 1. Wound size in both diabetic and normal animals was reduced by both processed and non-processed MAE. The rate of improvement in wound size by processed MAE in diabetic animals was significantly more than that of non-processed MAE. Non-processed MAE could not completely heal wound and mild injury was evident by histopathology examination. In mild injury wounds, the epiderm was thin in acidophilic background. Disturbed derm structure infiltrated by fat and edema was evident. Mononuclear cells were present. The vessels were dilated and bleeding was observed. In recovered cases, derm and epiderm were in normal structure (Figures 1-3).

Table1. Effects of drugs 1 and 2 on wound healing in diabetic and normal animals

Groups (type of drug)	Wound size on 7^{th} day	Wound size on 15^{th} day	Histopathologic result
Diabetic mice (Drug 1)	1.07	0.15[aa]	Mild injury
Diabetic mice (Drug 2)	0.73	0 [aa, bb]	Recovered
Diabetic mice (NS)	0.75	0.11 [aa]	Mild injury
Normal mice (Drug 1)	0.94	0 [aa]	Recovered
Normal mice (Drug 2)	0.45	0 [aa]	Recovered
Normal mice (NS)	0.53	0.12 [aa]	Mild injury

Drug 1= MAE without treatment with electromagnetic field radiation; Drug 2= MAE treated with electromagnetic field radiation for 3 minutes for 3 times; NS= normal saline. [aa] Difference between day 7^{th} and 15^{th} wound size is significant at $P<0.01$. [bb] Difference between Drug 1 and Drug 2 groups is significant at $P<0.01$.

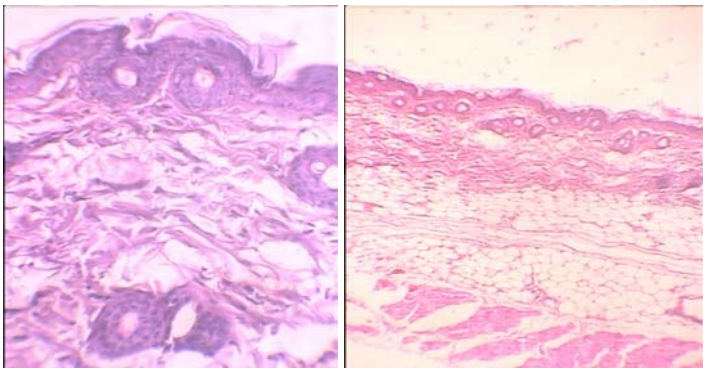

Figure 1. Histograms from derms of diabetic animals treated by saline. The epiderm is thin acidophilic background. Disturbed derm structure is infiltrated by fat and edema is evident. Mononuclear cells are. The vessels are dilated and bleeding is observed.

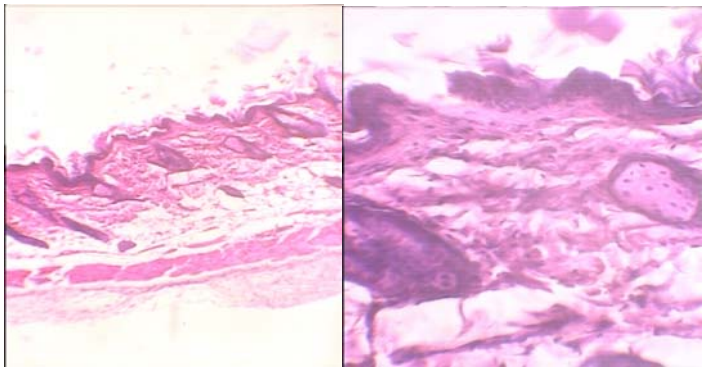

Figure 2. Histograms from derms of diabetic animals treated by normal MAE. Comparing with the figure 1, mild injury is still observed.

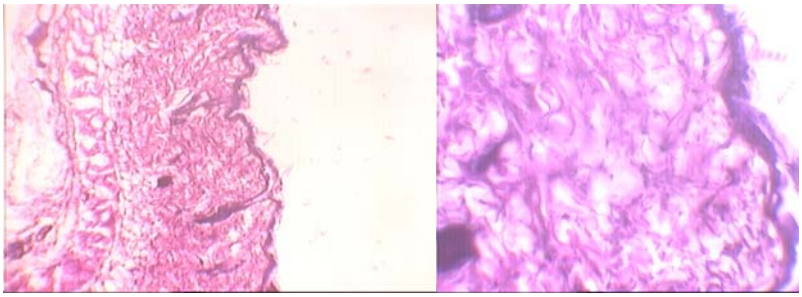

Figure 3. Histograms from derms of diabetic animals treated by electromagnetically-processed MAE. Comparing with figure 1, injury has been almost recovered and both derm and epiderm are in normal structure.

Discussion

Taking results together, it is evident that ANGIPARS is beneficial in wound healing but electromagnetical processing increases its effectiveness.

Data confirm that wound size significantly decreases after 7-day topical application of MAE in diabetic mice. In agreement with this result, the recent clinical trial showed that oral administration of ANGIPARS in diabetic subjects leaded to at least 50% reduction in wound size after 8 weeks[14]. Another clinical trial indicated a primary wound healing 2 weeks after topical application of ANGIPARS and a 70% improvement in wound surface area after 6 weeks in patients with diabetic foot ulcers[16]. In addition, co-administration of intravenous and topical ANGIPARS reduced human pressure ulcer after four weeks administration[17,18].

Regarding the key role of oxidative stress in pathogenesis and complications of diabetes, there is no doubt that compounds with strong antioxidant potentials can be beneficial[20]. *Melilotus officinalis* has been found to have strong antioxidant components such as oleanene glucuronide[21], flavonoids, and coumarins[8].

Diabetic foot ulcer is included in chronic wounds such as neuropathic ulcers, including neuropathic forefoot ulcers, diabetic pressure ulcers or diabetic venous ulcers. Therefore, it seems that MAE can be used for treatment of bed sores, which affect people who stay in one position for an extended period of time for any reason. Interestingly, the present study confirmed that MAE provides full wound healing with accelerated wound closure, and unexpectedly it improves the quality of the tissue in the healing wound with very efficient hair growth on the scars. Obtaining healthier scar tissue ensures the lower rate of ulcer recurrence in the future.

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