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The therapeutic benefits of *Melittis melissophyllum* extracts in wound healing

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Summary: Both aqueous and alcoholic extracts of the plant *M. melissophyllum* have significant therapeutic potential in the treatment of various types of superficial wounds. The topical application of these extracts was shown to relieve inflammation, increase cellular proliferation, promote collagen synthesis at the wound site and result in faster wound closure than experimental controls using an induced burn rodent study and induced diabetic wounds. These properties suggest its potential benefit as a drug product to treat diabetic foot ulcers, pressure ulcers, burns and other problematic skin conditions.

Introduction: The process of wound healing is comprised of a continuous sequence of inflammation and repair, in which epithelial, endothelial, inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains, interact to restore a semblance of their usual discipline and having done to resume their normal function. The formation of granulation tissue into an open wound allows the re-epithelialization phase to take place, as epithelial cells migrate across the new tissue to form a barrier between the wound and the external environment. Collagen deposition is an important component of this process, because it increases the strength of the wound. Also, cells involved in inflammation, angiogenesis and connective tissue construction attach to grow and differentiate on the collagen matrix established by fibroblasts. Successful wound healing is dependent upon various cell types, molecular mediators and structural elements. Among the factors that contribute to non-healing chronic wounds are diabetes, venous or arterial disease, infection, metabolic deficiencies of old age and applied chemicals from delivery systems. It was recently discovered that *M. melissophyllum* extracts have high therapeutic activity in all stages of the wound healing process, relieve inflammation, accelerate cell proliferation and therefore shorten overall time to wound closure.

Materials & Methods: Experimental model used for study; male wistar rats, 208-300 g. In the study three groups of mammals (with 6 rats in each group) with open wounds were involved: Group-1: Control with no treatment; Group-2: A typical antiseptic agent was used, chlorohexidine in accordance with a standard protocol regarding treatment of an infected wound; Group-3: The herbal preparation with *M. melissophyllum* extracts was used. Phagocytic activity of white blood cells was assessed using Phagocytic Index (PI) and an amount of phagocytes (PF). Diabetes was induced with a single injection of alloxane 170 mg/kg b.w. Hyperglycemia was defined as a random blood tail glucose level which exceeded >16 mmol/l on the 10th day of experiment. Statistic data processing with GraphPadPrism v.5.

Results: Recent *in vitro* studies have demonstrated inhibitory effect on inflammation and limited cytotoxicity to human keratinocytes and dermal fibroblasts. Immunogenicity testing did not show any significant allergic response and further more assures cellular longevity. Histological experiments demonstrated the formation of normal epithelial layers on the wound surface as evidenced by 20% increase in collagen content in wound tissue. Herbal extract has comparable antimicrobial property to antiseptic agent, chlorohexidine. Extract inactivates bacteria and their toxin without any side effect. Evaluation of DNA biosynthesis increased ~3X relative to the control group and significant collagen deposits were observed in the wound tissue. Additionally, the drug treatment group showed a significant increase in strength of scar tissues in the incision wound model and also increased the strength of collagen tissue. In addition, it has been shown that the formation of multilayer cells in the upper regions of the wound enhanced barrier function for external invasion of microorganism and resulted in accelerated wound closure by 3-4 days with complete epithelialization on the 9th day vs. 12-13 day for control.

Conclusions: Natural materials are still considered ideal sources for treating a broad range of diseases and damage of epithelial tissue. *M. melissophyllum* extracts relieved inflammation, increased cellular proliferation, promoted collagen synthesis at the wound site as evidenced by the increase in DNA, protein, collagen content and tensile strength. This natural agent demonstrated promising results and improved wound healing and regeneration of the lost tissue by multiple mechanisms without any observance of toxic effect on either the immediate area of tissue damage or the full body. While these data are encouraging, there is a need for further scientific validation, chemical standardization and broad safety assessment of this plant in the area of wound healing. We looking for an opportunity to expand the scope of work around this plant species and further assess the chemical composition of the extracts and investigate its mechanism of action in wound healing and/or other skin conditions that may be relevant.

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