Effect of two homeopathic remedies at different degrees of dilutions on the wound closure of 3T3 fibroblasts in *in vitro* scratch assay

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ABSTRACT

Background: Since ancient times, preparations from traditional medicinal plants e.g. *Arnica montana*, *Calendula officinalis* or *Hypericum perforatum* have been used for different wound healing purposes. The aim of this study was to investigate the efficacy of the commercial low dilution homeopathic remedy Similasan® Arnica plus Spray, a preparation of *Arnica montana* 4x, *Calendula officinalis* 4x, *Hypericum perforatum* 4x and *Symphytum officinale* 6x (0712-2) and medium diluted SIM WuS (*Petroleum* 15x, *Arnica montana* 15x, *Calcium fluoratum* 12x, *Calendula officinalis* 12x, *Hepar sulfuris* 12x and *Mercurius solubilis* 15x; 1101-4), on the wound healing in cultured NIH 3T3 fibroblasts. Both remedies were from Similasan AG (Jönen, Switzerland) and prepared according the German Homoeopathic Pharmacopoeia (GHP) following descriptions 4a for *arnica*, 3a for *marigold* and *St. John's wort*, 2a for *comfrey*, 5a for *petroleum*, and 6 for *calcium fluoride*, *hepar sulfuris* and *mercurius solubilis*.

Materials and Methods: Cell proliferation, migration and wound closure promoting effect of the preparations (0712-2, 1101- 4) and their succussed solvents (0712-1, 1101-3) were investigated on mouse NIH 3T3 fibroblasts. Cell viability was determined by WST-1 assay, cell growth using BrdU uptake, cell migration by chemotaxis assay and wound closure by CytoSelect™ Wound Healing Assay Kit which generated a defined wound area. All assays were performed in three independent controlled experiments. In some experiments diluted unsuccussed alcohol (0712-3) was also investigated.

Results: Preparations (0712-1), (0712-2), (0712-3), (1101-3) and (1101-4) were investigated at decimal dilution steps from 1x to 4x. Cell viability was not affected by any of the substances and (0712-1) and (0712-2) showed no stimulating effect on cell proliferation. Preparation (0712-2) exerted a stimulating effect on fibroblast migration (31.7%) vs 15% with succussed solvent (0712-1) at 1:100 dilutions (p<0.001). Unsuccussed solvent (0712-3) had no influence on cell migration (6.3%; p>0.05). Positive control 2 ng/ml EGF increased migratory activity of cells by 49.8%. Preparation (0712-2) at a dilution of 1:100 promoted *in vitro* wound closure by 59.5% and differed significantly (p<0.001) from succussed solvent (0712-1), which caused 22.1% wound closure. Medium diluted remedy (1101-4) exerted accelerating effect on wound closure after 14h of treatment. Wounded area was closed by 20% with (1101-4) and 13% by (1101-3) compared to untreated control. Succussed solvent (1101-3) caused about 23% and the remedy (1101-4) about 30% wound closure after

24h. Remedy (1101-4) and succussed solvent (1101-3) modestly stimulated cell growth at dilutions 1:100 and 1:1000 by about 25% and 15%, respectively. No statistically significant differences between preparations 1101-3 and 1101-4 could be detected.

Conclusions: Our results demonstrate that the Similasan® Arnica plus low dilution homeopathic remedy exerted wound healing potential, which is a result of increased ability of fibroblasts to migrate without affecting cell proliferation. Medium diluted preparation SIM WuS exerted stimulating effect on the wound closure accompanied by a cell proliferating effect.

Used *in vitro* wound closure test was sensitive enough for low dilutions preparation, however for medium diluted preparation despite of a trend, no significant differences could be detected.

Key words: wound healing, 3T3 fibroblasts, homeopathic remedy, *arnica*, *calendula*

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