***Keywords***: Individualised homeopathy, Meta-analysis, Randomised placebo-controlled trials, Systematic review

## Effects of homeopathic *Arnica montana* on gene expression of human macrophages-results of quantitative real-time PCR

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***Introduction***: *Arnica montana* is a plant belonging to the Compositae family and one of the most popular medications used in complementary and homeopathic medicine to treat inflammation, wounds, hematoma, contusion and pain. Recently has been pointed out the double role of the sesquirterpenic lactone helenalin in the inhibition of the transcription factor NF-KB directly targeting p65 and in the gene suppression of the same subunit. This study considers the changes due to different homeopathic dilutions in gene expression of several cytokines, chemokines and receptor by real-time PCR technique in monocyte/macrophage cellular model.

***Methods***: The effect of *Arnica m.* on gene modulation of human monocytes (THP-1 cell line) was analyzed with RT-ARRAY PCR technique. THP-1 cells differentiated into activated macrophages by phorbol-12-myristate-13-acetate (PMA) for 48 h were challenged with different homeopathic dilutions of *Arnica m.* (2c, 3c, 5c, 9c and 15c diluted/dynamized in water, with 0.03% ethanol final concentration) and with control solution (water with 0.03% ethanol). Drug-treated and untreated macrophages were incubated for 24 h in the absence and in the presence of 10 ng/ml *E. Coli* lipopolysaccharide (LPS). Total RNA was extracted and retro-transcribed into cDNA to quantify the relative amount of gene trascripts (SYBR Green dye) in treated cells respect to placebo (DDCt method).

***Results and discussion***: The treatments with *Arnica m.* homeopathic dilutions in cell cultures without LPS induced a significant changes in gene expression modulation for the CCL2 (Freg = −40%), IL-1B (Freg = −50%) and TNF-a (Freg = −25%), compared with vehicle solution. The effect was not linearly related to dilution/dynamization, showing a pattern of down-regulation genes in all dilutions tested, with the exception of 15c. Different patterns were observed in the presence of LPS, where only BMP2 gene resulted slightly up-regulated (Freg = +20%). Our findings are compatible with a mild modulation of inflammatory process by homeopathic dilutions/dynamizations of this plant, even if further studies are needed to clarify the molecular targets.

## Investigation of effects of highly diluted substances in periodontal inflammation using flow cytometry analysis – a pilot study

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***Background***: Homeopathic drugs are applied in complementary treatment of periodontal inflammation. However, less is known about the basic working principles of highly diluted remedies in such chronic inflammatory conditions. We therefore aimed at investigating the in vitro effects of highly diluted substances in periodontal inflammation by using fluorescence-activated cell sorting (FACS) analyses.

***Material and methods***: CD4+ lymphocytes were extracted from blood samples of three patients suffering on chronic aggressive periodontitis and three matched healthy volunteers and mixed with potentized diluted aqueous extract (D12 and C200) from *Mercurius solubilis*, *Silicea*, *Sulphur*, *Tuberculinum*, or a placebo. Activation patterns were then analyzed by means of the density of temporary expression of surface markers CD25 and CD45R0 in FACS. Statistical analyses were performed using descriptive statistics and correlation analysis.

***Results***: In total, the potentized aquaetous extracts yielded to a variety of effects both with respect to the lymphocytes of healthy volunteers vs. periodontitis patients, as well as to the potencies used (D12 vs. C200). Only *Mercurius* D12, *Silicea* C200 and *Sulfur* D12 showed similar activation patterns of CD25 and CD45R0 markers while all other substances did not provide concordant response. Of these three substances, *Sulfur* D12 showed the highest change in expression of CD45R0 markers in the healthy volunteers (+35.39%) as well as in patients (+36.47%). This was also confirmed in the analysis of CD25 expression.

***Conclusion***: Discussion about the basic working principles of highly diluted substances is still vital and leads to controversies in the scientific discussion. Although conclusions are limited due to low sample size, our pilot study was able to reproduce former results on lymphocyte migration activity with *Sulfur* D12.

***Keywords***: CD 4 lymphocytes, FACS analyses, Chronic aggressive periodontitis, *Sulfur* D12