

Silvana Marques de Araújo\*,  
Camila Fernanda Brustolin, Paula Fernanda Massini,  
Neide Martins Moreira,  
Carlos Edmundo Rodrigues Fontes  
and Denise Lessa Aleixo

*Universidade Estadual de Maringá, Brazil*

\*Correspondence: Prof Silvana Marques de Araújo,  
Universidade Estadual de Maringá, Brazil.  
E-mail: [smaraujo@uem.br](mailto:smaraujo@uem.br) (S.M. de Araújo)

Research using highly diluted drugs has advanced significantly with increasing number of consolidated research groups. This work evaluates the influence of highly diluted *Lycopodium clavatum* or *Phosphorus* in Wistar rats infected by *T. cruzi*. The experiment was conducted as a blind, controlled and randomized by draw assay and was approved by university ethical committee. 75 male rats (*Rattus norvegicus*, Wistar lineage), 45 days old, intraperitoneally inoculated with  $5 \times 10^6$  *T. cruzi* Y-strain blood trypomastigotes were divided into three groups: IC (infected control group, treated with 7% hydroalcoholic solution), Ly (infected treated with *Lycopodium clavatum* 13CH), Phos (infected treated with *Phosphorus* 13 CH). All treatments were offered ad libitum on the second day before the infection, and on the second, fifth and seventh day after infection, provided for 16 consecutive hours. Evaluated parameters: weight, temperature, water and food intake, amount of excreta, intestinal length and diameter, hair aspect, stool consistency, heart and respiratory rates, pre-patent period, parasitemia peak, total parasitemia, evaluation of myenteric neurons, inflammatory infiltrate and cytokines production. Data were statistical compared. *Lycopodium* and *Phosphorus* have significant beneficial effects on the clinical evolution of the treated animals. No significant difference was observed for any parasitological parameter evaluated. Ly and Phos groups showed protection of distal colon neurons numbers. In the heart, liver and intestine animals treated with *Lycopodium* and *Phosphorus* showed significant less inflammation compared to IC. In striated skeletal muscle, *Phosphorus* animals showed the number of inflammatory foci higher than IC. In a sequential evaluation, IL1- $\alpha$ , IL1- $\beta$ , IL4, IL6, IL10, IL12, TNF- $\alpha$ , IFN- $\gamma$  and GM-CSF levels varied significantly different for *Lycopodium clavatum* and *Phosphorus*. The homeopathic treatment with *Lycopodium clavatum* or *Phosphorus* medicines (13CH) promoted, in a different way, beneficial effects on several parameters evaluated in *T. cruzi* infection of Wistar rats. The treated groups establish balance of host-parasite relation differently, with lower cell and tissue damage to the infected host. *Lycopodium clavatum* and *Phosphorus* modifies the animals' immune response, promoting less inflammation and protecting the intestine, preserving the myenteric neuronal population.

**Keywords:** Trypanosoma cruzi, Clinical evaluation, Myenteric neurons, Cytokines, Homeopathic medicine, *Lycopodium clavatum*, *Phosphorus*

## Systematic review and meta-analysis of randomised, placebo-controlled, trials of individualised homeopathic treatment

Robert T Mathie\*, Michel Van Wassenhoven,  
Jennifer Jacobs, Menachem Oberbaum, Joyce Frye,  
Raj K Manchanda, Helmut Roniger, Flávio Dantas,  
Lynn A Legg, Jürgen Clausen, Sian Moss,  
Jonathan RT Davidson, Suzanne M Lloyd, Ian Ford  
and Peter Fisher

*British Homeopathic Association (BHA), UK*

\*Correspondence: Dr Robert T Mathie, British Homeopathic Association (BHA), UK.  
E-mail: [rmathie@britishhomeopathic.org](mailto:rmathie@britishhomeopathic.org) (R.T. Mathie)

**Background:** The BHA's programme of systematic reviews of randomised controlled trials (RCTs) of homeopathy distinguishes key attributes of study design and intervention: placebo controlled *cf.* other-than-placebo controlled; individualised *cf.* non-individualised homeopathy; treatment *cf.* prophylaxis.

**Objective:** This presentation centres on the hypothesis: For the broad spectrum of medical conditions that have been researched using RCTs, the main outcome of an individualised homeopathic treatment approach using homeopathic medicines is distinguishable from that of the same approach using placebos (i.e. individually prescribed homeopathic medicines have specific effects). The impact of internal validity (risk of bias, RoB) and model validity (MV) informed the detailed interpretation of results.

**Methods:** 31 papers (reporting a total of 32 RCTs) were eligible for systematic review. For each trial, the separate ratings for RoB and MV were merged to obtain a single overall designation ('high', 'moderate', 'low' or 'very low' quality). The identified main outcome measure was extracted, if possible, for each trial and used in meta-analysis.

**Results:** Combining assessment of MV and RoB identified 3 trials of 'high quality', 8 of 'moderate quality', 18 of 'low quality' and 3 of 'very low quality'. This hierarchy was little different from that attributed to RoB alone, and so the meta-analysis findings were essentially unchanged by accommodating MV: a small, statistically significant, pooled odds ratio (OR) favouring homeopathy (mean = 1.53; N = 22) that is robust to sensitivity analysis based on best evidence (mean OR = 1.98; N = 3). There was no association between a trial's MV and RoB or direction of treatment effect.

**Discussion:** Accommodating MV in the quality appraisal of RCTs does not alter the conclusion that individually prescribed homeopathic medicines may have small, specific, treatment effects. This conclusion reflects evidence in individualised homeopathy across a broad spectrum of medical conditions and thus transcends condition-specific analysis.

**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

D Oliosio, M Marzotto, C Bonafini and P Bellavite

University of Verona, Italy

Correspondence: D.ssa Debora Oliosio, PhD, University of Verona, Italy. [debora.oliosio@univr.it](mailto:debora.oliosio@univr.it)

**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 sesquiterpenic 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 transcripts (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- $\alpha$  (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

C Raak\*, F Schaupp, F Entschladen, T Ostermann, G Gassmann, K Büchel and A Friedmann

Institute of Integrative Medicine, Witten/Herdecke University, Germany

\*Correspondence: Christa Raak, BSc, Institute of Integrative Medicine, Witten/Herdecke University, Gerhard-Kienle-Weg 4, 58313, Germany. E-mail: [Christa.raak@uni-wh.de](mailto:Christa.raak@uni-wh.de) (C. Raak)

**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 aqueous 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