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Authors: Bianka Lutz, Iris Heer, Ramona Katzensteiner, Christa Raak, Ursula Wolf, Peter Heusser, Michael Frass, Stephan Baumgartner

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ACCEPTED MANUSCRIPT

Development of a whole plant bioassay to test effects of potentized calcium carbonate in pillule formulation

Bianka Lutz [1,2,3], Iris Heer [1], Ramona Katzensteiner [4], Christa Raak [2], Ursula Wolf [1], Peter Heusser [2], Michael Frass [4], Stephan Baumgartner [1,2]

[1]Institute of Complementary Medicine, Fabrikstrasse 8, University of Bern, 3012 Bern, Switzerland

[2]Institute of Integrative Medicine, University of Witten/Herdecke, Gerhard-Kienle-Weg 4, 58313 Herdecke, Germany

[3]Research Institute for Organic Agriculture, Ackerstrasse, 5070 Frick, Switzerland

[4]Department of Medicine I, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria

Correspondence: Stephan Baumgartner, stephan.baumgartner@uni-wh.de

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Summary

Objectives: From a pharmaceutical point of view, we see a need to develop stable preclinical test systems to identify and investigate effects of potentized remedies as used in Anthroposophic Medicine and Homeopathy. We evaluated a plant bioassay regarding its capacity to distinguish homeopathic remedies from placebo, applied as sucrose pillules.

Methods: Pea seed (*Pisum sativum* L) was soaked for 24 hours in water with dissolved homeopathic or placebo pillules, or in water only. Shoot length was measured 14 days after planting and treatment groups were compared by analysis of variance (ANOVA). The stability of the system was validated by systematic negative control experiments.

Results: The system is suitable to test a common application form – sucrose pillules – of a potentized preparation without influence of the pharmaceutical carrier substance. A screening of 13 potentized preparations revealed *Calcium carbonicum* to affect pea shoot growth ($p<0.05$). Three independent series of main experiments were performed with potentized *Calcium carbonicum* to assess reproducibility. Meta-analysis of all data revealed significant effects of *Calcium carbonicum* 12c and 30c on pea shoot growth ($p<0.05$), which were however dependent on the date of experiment and/or the experimental series.

Conclusions: Potentized *Calcium carbonicum*, applied as sucrose pillules, influenced pea shoot growth in the assay investigated. However, due to the small effect size and due to the modulation of the effects by still unknown external factors, further optimization of this bioassay is necessary to be used in pharmaceutical quality control or in investigating the biological or pharmaceutical mode of action of potentized preparations.

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