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ORIGINAL PAPER

Reproducibility of the effects of homeopathically potentised *Argentum nitricum* on the growth of *Lemna gibba* L. in a randomised and blinded bioassay

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Background: **A previous study reported a significant statistical interaction between experiment date and treatment effect of *Argentum nitricum* 14x−30x on the growth rate of duckweed (*Lemna gibba* L.). The aim of the present study was to investigate the stability of the test system and intra-laboratory reproducibility of the effects found.**
Methods: **Duckweed was treated with *A. nitricum* potencies (14x−30x) as well as succussed and unsuccussed water controls. The outcome parameter area-related growth rate for day 0−7 was determined by a computerised image analysis system in two series of independent randomised and blinded experiments. Systematic negative control (SNC) experiments were carried out to investigate test system stability. Statistical analysis was performed with full two-way analysis of variance (ANOVA) and protected Fisher's Least Significant Difference (LSD) test.**
Results: **In the first repetition series we found a significant treatment effect (p = 0.016), while in the second series no effect was observed. The negative control experiments showed that the experimental system was stable. An *a posteriori* subgroup analysis concerning gibbosity revealed the importance of this growth state of *L. gibba* for successful reproduction of the statistically significant interaction in the original study; flat: no interaction (p = 0.762); slight gibbosity: no interaction (p = 0.356); medium gibbosity: significant interaction (p = 0.031), high gibbosity: highly significant interaction (p = 0.005).**
Conclusions: **With the original study design (disregarding gibbosity status of *L. gibba*) results of the original study could not be reproduced *sensu stricto.* We conclude that the growth state gibbosity is crucial for successful reproduction of the original study. Different physiological states of the test organisms used for bioassays for homeopathic basic research must carefully be considered.** *Homeopathy* (2017) ■, 1−10.

Keywords: Homeopathy; Plants; Duckweed; Silver nitrate; Reproducibility

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Introduction

Stability of test systems and reproducibility of results is one of the most challenging issues in homeopathic basic research.[1−3] Several groups aimed to develop simple and reliable preclinical experimental systems to assess the effects of homeopathic treatments and have published

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several high quality basic research studies,[4–7] which revealed significant specific effects of homeopathic remedies.[8–12] However, only few trials have been published that investigated the reproducibility of earlier results. Some studies aiming to reproduce the results of original trials could not confirm the original findings.[13,14] This phenomenon implies that it is essential to identify confounders and factors that may influence results of experiments in basic homoeopathic research in order to be able to improve stability and validity of test systems.

Regarding plant based test systems, such influencing factors may consist, for example, of seed quality[15,16] or specific plant growth stages. By investigating reproducibility of the study by Scherr *et al.*[12] regarding the effects of potentised gibberellic acid on growth of duckweed (*Lemna gibba* L.), Majewsky *et al.* identified gibbosity, a specific growth state, of *L. gibba* as an essential condition for duckweed's responsiveness to homeopathic treatment with gibberellic acid potencies.[17]

Duckweed (*Lemna gibba* L.) is a small, monocotyledonous water plant, which grows vegetatively and exponentially under specific laboratory conditions by developing daughter fronds (leaf-like structures) out of the pouches of the mother fronds. This enables working with a high number of genetically identical cultures at the same time or over a long period of time. *L. gibba* can be distinguished from all other *Lemnaceae* species by the occurrence of gibbosity. Gibbosity is a characteristic swelling of the water-immersed side of the fronds (Figure 1), which develops under certain environmental conditions by enlargement of the air chambers in the fronds.[18] In nature, gibbosity may be advantageous in situations of competition with other *Lemna* species. Because of its convex lower side *L. gibba* is able to grow over the flat fronds of other *Lemna* species,[19–21] and additionally, absorption of nutrients is improved by the enlarged surface of gibbous fronds.[22] In nature and under laboratory conditions the plant hormone ethylene was found to be responsible for inducing gibbosity.[23,24] The fact that endogenous ethylene synthesis increases in situations of overcrowding, when fronds are pushed together,[24,25] supports the interpretation of gibbosity as competition strategy.

Due to its sensitivity to organic and inorganic substances, duckweed is used as research organism in ecotoxicology, physiology and genetics. Scherr *et al.* adapted a standardized ecotoxicological test[26,27] for the use in homeopathic basic research and investigated the influence of 12 substances, potentised from 14x to 30x, on the growth rate of *L. gibba*.[11] In a subsequent study[12] Scherr *et al.* repeated experiments with four test substances in homeopathic preparation (gibberellic acid, kinetin, *Argentum nitricum* and an extract of *Lemna minor*). Whilst gibberellic acid exerted a consistent treatment effect on the growth of duckweed in the course of five independent experiments, *A. nitricum* induced a response to the different potency levels that varied between experiments (decrease, increase or no difference in growth compared to the water control).

The aim of the present study was to investigate whether the results of Scherr *et al.*[12] with potentised *A. nitricum* had intra-laboratory reproducibility. In an additional *a posteriori* subgroup analysis, we analysed the relevance of the specific growth stage gibbosity on the experimental outcome, since in another investigation it was observed that gibbosity plays a decisive role for responsiveness of duckweed to potentised gibberellic acid.[17]

Materials and methods

For repetition of the experiments by Scherr *et al.*[11,12] the same *L. gibba* bioassay was used. Cultures of *Lemna gibba* L. were clones from the original strain, and we worked with the same test design and potentisation techniques. Statistics, laboratories, materials, and chemicals were identical to the ones used in the study by Scherr *et al.* The present experiments differed only regarding date and person who performed the experiments, including preparation of *A. nitricum* potencies. In the following we give an abridged account of materials and procedures used, since they were described in detail by Scherr *et al.*[11,12,27] The present report was prepared according to current publication guidelines for homeopathic basic research.[28]

Preparation of homeopathic samples and controls

Argentum nitricum was obtained as aqueous 4x (Weleda AG, Arlesheim, Switzerland) and potentised from 5x to 30x by the multiple-glass method. In order to prepare 5x, 3 ml *A. nitricum* 4x was added to 27 ml of distilled and autoclaved water (in the following called water) and succussed by hand for 2 min, shaking in a horizontal line at a rate of approximately 2 Hz at room temperature. Producing further potencies, 30 ml of the respective previous potency level was potentised with 270 ml of water, using thoroughly cleaned glass vessels (500 ml, Duran®, Schott, Mainz, Germany). Additionally, two controls were prepared for every experiment. Control c0 was not succussed, being prepared by pouring 300 ml of water into a potentisation bottle without further agitation. Control c1 was prepared with 300 ml water, succussed once in the same way as the potencies, in order to control the influence of ions that dissolve from the glass bottle during succussion as well as other effects attributable to the succussion process. We did not use potentised controls, since Witt *et al.*[29] observed an equilibrium in ion concentration after the first succussion process, which did not increase during further dilution and succussion. Additionally we wanted to avoid a possible effect of potentisation on the water control.[30] All potencies and controls were freshly prepared on the day of every single experiment from the same batch of distilled and autoclaved water.

Blinding

After preparation, bottles with potencies and controls were manually blinded and randomised with a double letter code (AA, BB, CC...) by a person not being involved in the experiments. The corresponding codes were kept closed

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